







METHODS OF ANALYSIS  
A O. A. C.





METHODS OF ANALYSIS, A.O.A.C., 4th EDITION, 1935

ERRATA AND REFEREES' EMENDATIONS\*

Page	Section
18,	5, note under Hdg. . . . . Delete first word, "Not."
30,	44(a), line 9. . . . . Insert "After 15 min." before "Filter."
126,	16, line 11. . . . . Change " $\text{KClO}_3$ " to " $\text{KClO}_4$ ."
135,	46. . . . . Change "39" to "38."
149,	6, line 5. . . . . Change "5" to "5(a)."
	8, line 2. . . . . Enclose " $A \times 2.0665 + E$ " in parentheses.
	last line. . . . . Change "6" to "7(a) or (c)."
150,	10, line 1. . . . . Delete "to remove $\text{CO}_2$ ."
	18, line 2. . . . . Change "evaporate to dryness" to "concentrate to sirupy consistency."
151,	20, 3rd line from bottom. . . . . Change "thymolphthalein" to "phenolphthalein."
152,	21, line 1. . . . . Delete "necessarily."
	27. . . . . Change to "Place a 100 cc sample in a Pt dish, add 200 cc of a 5% soln of $\text{Na}_2\text{CO}_3$ and proceed as directed under XII, 34-37."
	28, line 4. . . . . Change "19" to "23" and add "See also XVI, 25."
160,	51, line 6. . . . . Change "prepared" to "finely ground."
161,	51, line 3. . . . . Change "inversion" to "conversion."
	55, line 11. . . . . Change "inverted" to "converted."
168,	30(c). . . . . Change "sulfonate" to "disulfonate."
175,	24, note, last line. . . . . Change "Table 24" to "XLII, Table 20."
195,	35, 36. . . . . Change "11" to "12."
212,	29, line 2. . . . . After "add" insert "30 cc. of $\text{C}_2\text{H}_5\text{OH}$ and."
	line 5. . . . . After "separatory funnel" add "(glass stopcock lubricated with water)."
305,	28. . . . . Change "72" to "79."
338,	49, line 6. . . . . Change "(h)" to "(f)."
347,	44, line 8. . . . . Change " $(p\text{H } 3.0-4.4)$ " to " $(p\text{H } 2.5-3.0)$ ."
	line 9. . . . . Change "20-30 cc" to "10 cc."
348,	47, line 3. . . . . Add "2-4" hrs." to sentence in parentheses.
365,	41, line 2. . . . . Change "32, 33" to "34, 35."
378,	379, 13(j) and (p). . . . . Insert "phosphate-free" before "KCN."
423,	line 2. . . . . Add "If the melting point of the glycerides plus twice the difference between the melting point of the glycerides and the melting point of the fatty acids, is less than $73^\circ$ , the lard is regarded as adulterated."
439,	29, last line. . . . . Delete subscript "s."
460,	79, last line. . . . . Change "26" to "27."
463,	5. . . . . Transpose Hdg. "By Means of a Spindle, etc." and the preceding note in parentheses.
464,	6, line 4. . . . . Change "LXII" to "XLII."
496,	Ref 35. . . . . Add "Trans. Roy. Soc. Can., 1919, Sec. III, 221."
	Ref. 38. . . . . Add "J. Ind. Eng. Chem., 5, 741 (1913); 8, 331 (1916); and J. Assoc. Official Agr. Chem., 4, 435 (1931) and delete "J. Am. Chem. Soc., 28, 435 (1906)."
500,	22, line 2. . . . . Change "33 and 35" to "34."
558,	44, line 13. . . . . Change "0.0043" to "0.00403."
619,	last column, line 12. . . . . Change "0.80" to "0.08."
626,	column 7, line 17. . . . . Change "29.6" to "26.9."

\* Reprinted from *J. Assoc. Official Agr. Chem.* May, 1937. See also report on "Changes in Methods."



OFFICIAL AND TENTATIVE

# METHODS OF ANALYSIS

OF THE

## ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS

EDITORIAL BOARD  
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H. A. LEPPER, and MARIAN E. LAPP

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FOURTH EDITION, 1935

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ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS  
AT WASHINGTON, D.C.

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ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS

The methods of the Association were also copyrighted in 1916, when they  
were published in the Journal of the Association of  
Official Agricultural Chemists

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## PREFACE TO FOURTH EDITION

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The fourth issue of *Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists* ("Methods of Analysis" or "Book of Methods") is presented to our members and to the public in accordance with the plan to publish a revision every five years. This book continues to grow in size and in the diversity of its subject-matter in harmony with the widening horizon of official chemists, who compose the Association. The philosophy of the Association, however, remains the same as in the beginning, and it is very aptly set forth by Dr. Harvey W. Wiley in the introduction to the first edition, which is herewith reprinted.

The general arrangement of subject matter follows the plan adopted for the second and third editions. Certain unsatisfactory methods have been omitted, for instance, the method for the determination of fluorine in baking powders. No general method for this determination has been included, since no satisfactory method has been perfected, although specific procedures for its determination under insecticides and waters have been presented as tentative methods. This subject is being intensively studied by the members of the Association and others, and those interested should refer to *The Journal* of the Association for the latest developments. The methods for beers, wines and distilled liquors, which constitute Chapter XVII in the third edition, have been assembled in three chapters entitled, respectively, Malt Beverages, Sirups and Extracts, and Brewing Materials; Wines; and Distilled Liquors. Two chapters of the third edition, i.e., XIII Fibers, and XIV Paper and Paper Materials, have been deleted because at the present time the work on these materials is not considered to be of sufficient importance to enough members of our Association to warrant cooperative referee work, and further because such methods are being satisfactorily studied elsewhere.

Attention is called to a few of the additions to the methods which have special significance. In Chapter I, Soils, the method for the determination of selenium is timely, as is also the method for the determination of the acid-forming and non-acid-forming quality of fertilizers, in Chapter II, Fertilizers, because these subjects have assumed special importance during the last two years. The method for the determination of the phenol coefficient of disinfectants, included in Chapter VI on Insecticides and Fungicides, is an example of a departure into a field of more unusual methods. Chapter XII, Plants, has been materially revised and enlarged, and now

includes a method for the determination of lignin. Methods have also been added under the chapter heading "Nuts and Nut Products."

The method for the determination of lead given in the chapter on Baking Powders has been deleted. Only the comprehensive method for lead in Chapter XXIX, Metals in Foods, has been included. The vitamin D assay by preventive biological test in Chapter XXVII, Grain and Stock Feeds, should be noted, as should also the revised and rearranged Chapter XXXIV, Sugars and Sugar Products.

Another innovation which should later make a valuable addition to the book is Appendix I, which presents methods for the preparation and standardization of solutions. At present only procedures for hydrochloric acid and alkali are given, but with the appointment of a referee on this subject other material will soon be available. A revised and enlarged section on definitions of terms and interpretation of results on fertilizers and liming materials constitutes Appendix II.

Since the issuance of the third edition, in 1930, the editorial work and the financial responsibilities of the Association have greatly increased. In 1929, upon recommendation of the Executive Committee, the Association organized an Editorial Board, composed of the Editorial Committees of *Methods of Analysis*, of *The Journal*, and of *Principles and Practice of Agricultural Analysis*. The Secretary-Treasurer of the Association was designated Chairman and Executive Officer of the Editorial Board. The Association named as the Editorial Committee for the fourth edition of *Methods of Analysis*, E. M. Bailey (Chairman), L. E. Warren, J. W. Sale, G. G. Frary, H. A. Lepper, and Marian E. Lapp. The revision of these methods is a serious and laborious task. Their official character and their acceptance in private and public litigation as the standard methods of analytical procedure place upon the Association a grave responsibility, which challenges the resourcefulness and industry of every referee and associate referee to keep the methods up to the highest point of accuracy and up to the minute in adaptability to current problems. Each member of the Editorial Committee has rendered excellent service in the production of the fourth edition. To Dr. Bailey, the Chairman, and to Miss Lapp, the Executive Secretary, the thanks of the Association are especially due. The Committee, however, recognizes and the members of the Association should recognize, that the strength of our Association rests upon the solid foundation of our unique system of investigation of methods, enthusiastically and generously supported by our referees, associate referees, and collaborators. Among those rendering special service in this revision should be mentioned C. H. Badger, R. T. Baleh, G. L. Bidwell, V. B. Bonney, C. A. Browne, I. D. Clarke, J. F. Clevenger, O. L. Evenson, H. J. Fisher, G. S. Fraps, R. W. Frey, J. J. T. Graham, V. E. Grotlich, B. G. Hartmann, H. D. Haskins, H. P. Holman, C. F.

Jablonski, G. S. Jamieson, R. H. Kerr, C. S. Ladd, J. A. LeClerc, R. E. Lothrop, J. S. McHargue, W. H. MacIntire, J. A. Mathews, L. C. Mitchell, A. E. Mix, V. E. Munsey, A. G. Murray, E. L. Pepper, S. C. Rowe, W. H. Ross, C. F. Snyder, L. S. Walker, W. B. White, H. J. Wichmann, J. B. Wilson, W. O. Winkler, and O. B. Winter.

It is believed that the fourth edition of *Methods of Analysis* deserves and will receive from its numerous readers as hearty approval as was accorded previous editions.

W. W. SKINNER

*Secretary-Treasurer of the Association of  
Official Agricultural Chemists,  
and Chairman, Board of Editors*

Washington, D.C.,  
December 31, 1935

## PREFACE TO THIRD EDITION

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The third issue of *Methods of Analysis* or *Book of Methods*, which are the abbreviated names of this publication, is offered to the members of the Association of Official Agricultural Chemists and to the public with a confidence that it will be as favorably received as were the previous editions. That this book of methods as originally conceived and executed fulfills the aims of its sponsors and meets the needs of a large group of official and control chemists is evidenced by the continued and unexpected demand which exhausted—six months before this edition was ready for distribution—the second edition of five thousand copies. The functions of the book and the philosophy of the development of the methods have been stated in the prefaces to former editions. These prefaces are reprinted here for reference purposes.

Although the arrangement is similar to that of the first and second editions, the changes, the rearrangements of data, and the additions and deletions are worthy of mention. The subject matter is broadly grouped into two divisions, non-foods and foods. The first division includes chapters on Soils, Fertilizers, Liming Materials, Insecticides, etc., thus bringing together related subjects, while the later chapters, devoted to foods, are arranged in alphabetical order, e.g., Baking Powders, Beverages, Beers, Coffee, Cereal Products, etc. This permits of easy reference and better meets the needs of a laboratory handbook. Several independent chapters in former editions have been combined with chapters on related



subjects, for example, the chapter on Vinegars has been combined with that on Spices and Other Condiments and the chapter on Gelatine has been included under Meat and Meat Products. Beers, Wines and Distilled Liquors have been combined in one chapter. The title Feeding Stuffs has been changed to Grain and Stock Feeds.

An evidence of the progressive development of the work of the A.O.A.C. is the inclusion of new chapters on Caustic Poisons, Naval Stores, Paints, Radioactivity, and Eggs and Egg Products. Chapter headings have been assigned to such subjects as Sewage, Fibers, Paper and Paper Materials, Nuts and Nut Products, etc., indicating new lines of work contemplated by the Association. Most notable among these new subjects are Vitamines, Microchemical Methods, and Bacteriological Methods. The inclusion of these chapter headings, especially the last-named, may seem out of place, but the interests of the agricultural chemist have so broadened in recent years that he finds it necessary to be professionally equipped to deal effectively with all those matters which come within the purview of research and control chemists. Therefore it has seemed wise to provide an arrangement to meet future needs. Indeed, the concepts of the profession of chemistry, especially as they apply to activities of the official chemist, have of necessity been extended to include physics, microbiology, bacteriology, microscopy, engineering, public health, etc.

An innovation in this edition is the placing of chapter numbers on each side of the page at the top and the page number at the bottom. This arrangement has facilitated editing by making it possible to include all cross references in the manuscript.

As heretofore, the methods are classified as official and tentative. In addition, note is made of those tentative methods which have received first action as official. Second favorable action by the Association is necessary, however, before these methods are finally adopted as official. This classification is important to those who use these methods to support action before the courts, since they are accredited by the Secretary of Agriculture in law enforcement work and are also accepted by the States in regulatory activities. In this connection it should be understood that the methods given under each caption apply in general only to the materials mentioned therein.

That the third edition might not be unduly large owing to the volume of added material, and to make it possible to sell the book at the former price, the editors have resorted to abbreviations and contractions, and to certain forms of simplified spelling, as for example, cc for cubic centimeter, g for gram, soln for solution, temp. for temperature, m.p. for melting point, c.p. for chemically pure, and the elements and common chemicals are expressed by symbols or formulas, as Cl, Br, Zn, HCl, CaCl<sub>2</sub>, KMnO<sub>4</sub>, etc. In referring to the common acids and to ammonia the words "strong"

and "concentrated" have been eliminated, it being understood that unless otherwise noted these reagents are the full strength product. The letter "C" after degrees of temperature has been omitted, because unless otherwise stated all temperatures in this volume are expressed in degrees centigrade. All tables have been rechecked, new ones inserted, and many of the old tables have been reduced in size when by so doing their usefulness was not impaired. These changes have saved space equivalent to many pages of printed matter.

While logically no part of *Methods of Analysis*, the editors, by a vote of the Association, have included as an appendix the Definitions for Fertilizers.

The organization for the work of the third edition differed materially from that of the first and second editions. Upon the recommendation of R. E. Doolittle, Chairman of the Committee on Revision for both the first and second editions, the Executive Committee ordered the preparation and printing in *The Journal* and in separates of all changes that had been made in the methods in any one year. The purpose of this plan was to facilitate the compilation of these changes at the time of revision.

The Editorial Committee appointed by the Association for the third edition consists of W. W. Skinner, Chairman; J. A. LeClerc, J. W. Sale, L. E. Warren, G. G. Frary and Marian E. Lapp. The cheerful and generous help given to the committee by the various general referees, associate referees and former referees has made this revision possible. Among those who deserve special mention are the following: S. Alfend, C. H. Badger, E. M. Bailey, L. H. Bailey, R. T. Balch, G. L. Bidwell, V. B. Bonney, I. D. Clarke, P. A. Clifford, M. R. Coe, J. Davidson, O. L. Evenson, G. S. Fraps, W. C. Geagley, J. J. T. Graham, V. E. Grotlich, B. G. Hartmann, A. M. Henry, J. T. Keister, R. H. Kerr, J. C. Krantz, Jr., C. F. Jablonski, G. S. Jamieson, C. S. Ladd, H. A. Lepper, W. V. Linder, H. C. Lythgoe, W. H. MacIntire, J. S. McHargue, R. M. McHurin, A. R. Merz, V. E. Munsey, E. M. Nelson, A. E. Paul, W. H. Ross, G. C. Spencer, F. P. Veitch, H. J. Wichmann, J. B. Wilson and O. B. Winter.

The work of the committee was organized by the selection of J. A. LeClerc as administrative secretary and Marian E. Lapp as assistant. To the painstaking and diligent efforts of these two members of the Revision Committee special credit for the compilation and prompt issue of the 1930 edition is due.

W. W. SKINNER  
*Secretary, Association of Official  
Agricultural Chemists, and  
Chairman, Board of Editors*

Washington, D. C.  
December 31, 1930

## PREFACE TO SECOND EDITION

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The methods of the Association of Official Agricultural Chemists are unique in several respects. They are the outgrowth of continual critical collaborative trial or test participated in by a large number of workers and undertaken in order to establish the accuracy of analytical results. They are subjected to a scrutiny of phraseology to insure clarity, probably unequaled in developing any similar methods. They are formulated solely by responsible Federal and State officials acting together and thus are based on underlying principles of equity. The Association has always encouraged to the utmost the cooperation of representatives of interested industries, but it has jealously reserved the final formulation of its methods to official chemists. Consequently, these methods have attained an enviable position in the fields of activity occupying the attention of the Association and are accepted as authoritative in matters at issue before the courts, both Federal and State.

As a result of the collaborative investigations conducted under the referee system, deletions, additions, and revisions in the methods are constantly being made. From 1884 to 1894 the methods of analysis adopted by the Association were published each year, with the secretary's report of the proceedings of the annual meeting, as a bulletin of the Division of Chemistry of the United States Department of Agriculture. In 1895 the methods, brought up to date to include the changes sanctioned by the 1895 meeting, were printed as Division of Chemistry Bulletin 46, which was later revised to incorporate the changes subsequently made at the annual meetings up to 1899. The provisional methods for the analysis of foods, authorized by the 1901 meeting, were issued in 1902 as Bureau of Chemistry Bulletin 65. In 1907 the official and provisional methods as adopted by the Association up to that time were printed as Bureau of Chemistry Bulletin 107, which was revised in 1908. From 1903 until 1912 circulars giving the official changes in the methods were issued annually, soon after each meeting. In 1920 the Association published the methods of analysis, revised to November 1, 1919, in book form under the title "Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists."

This revision, authorized in 1922, contains all the changes in methods adopted at the 1919, 1920, 1921, 1922, and 1923 meetings, as well as the polarimetric methods for the determination of sucrose and the methods for the determination of moisture and ash in wheat flour adopted in 1924.

The general plan of the original book has been retained. The cross-

references, however, are to page and section, not to chapter and section, and the following changes in the text have been made: Two new chapters, one on agricultural liming materials and one on gelatin, have been added; the chapter on water has been expanded to include brines and salts; the methods for sugar products formerly included in the chapters, Foods and Feeding Stuffs and Saccharine Products, have been combined in a chapter entitled Sugars and Sugar Products; and the methods for stock feeds have been rearranged under the chapter heading Feeding Stuffs.

The Committee on Editing Methods of Analysis of the association, consisting of R. E. Doolittle (chairman), G. W. Hoover, W. H. MacIntire, A. J. Patten, B. B. Ross, and J. W. Sale, prepared this revision. Miss Marian E. Lapp, associate editor of *The Journal*, gave the committee valuable assistance and edited the manuscript. Referees, associate referees, and other members of the Association assisted in compiling and critically reviewing the work.

W. W. SKINNER  
*Secretary, Association of Official  
Agricultural Chemists*

January 1, 1925

## INTRODUCTION TO SECOND EDITION

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In the present publication, the technic of agricultural analysis is brought up to date. The most valuable contribution made to agriculture in the last forty years has been that of the standardization of the chemical and physical methods of research in agriculture by this Association. The importance of sound and accurate laboratory methods is not so highly appreciated as it should be. Such methods are of supreme value not only to the agricultural worker, but also to all research workers in every branch of science, and especially in chemistry. A striking illustration of this is seen in the work on the nature and relative weight of the atom. It is not appointed to every one to become a leading expert. There are many good artists but only a few masters. There are many notable workers in research, but only a few Curies, Ramsays, Millikans, Rutherfords, and Richards. It is as much method and delicacy of manipulation as vision and initiative that make the master.

This revision of methods improves the value of the instruments in the hands of the seeker of new facts and the explanation of new laws.

HARVEY W. WILEY

## PREFACE TO FIRST EDITION

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In presenting this revision of the official and tentative methods of analysis of the Association of Official Agricultural Chemists, it is appropriate to give a brief statement of the organization of the Association, its purpose, and the procedure by which the methods are adopted.

Membership in the Association is institutional and includes the State Departments of Agriculture, the State Agricultural Colleges and Experiment Stations, the Federal Department of Agriculture, and the Federal, State, and City offices charged with the enforcement of food, feed, drug, fertilizer, insecticide and fungicide control laws.

The Association was founded at Philadelphia, Pa., September 9, 1884, by the following representative agricultural chemists of that time, the organization being the result of a series of informal meetings held the immediately preceding years:

Prof. H. W. Wiley, Chemist of the Department of Agriculture, Washington, D. C.

Mr. Clifford Richardson, Assistant Chemist of the Department of Agriculture, Washington, D. C.

Mr. Philip E. Chazal, State Chemist of South Carolina.

Dr. Chas. W. Dabney, Jr., State Chemist of North Carolina.

Dr. W. J. Gascoyne, State Chemist of Virginia.

Dr. E. H. Jenkins, Connecticut Experiment Station.

Prof. John A. Myers, State Chemist of Mississippi.

Prof. H. C. White, State Chemist of Georgia.

Mr. C. DeGhequier, Secretary National Fertilizer Association.

Dr. Schumann, Dr. Lehmann, Mr. Gaines and others.

At the first meeting methods for the determination of ammonia, phosphoric acid and potash in commercial fertilizers were adopted and work was begun for the perfection and adoption of methods for the entire range of agricultural chemistry. Later the passage of food and drug and insecticide and fungicide control legislation by the States and by the Federal Government made it necessary to extend the scope of the Association's activities for the reason that the Association methods were designated as the official methods for the enforcement of such legislation as well as for the control of feeds and fertilizers by the various states.

To attain the aims of the Association for a set of accurate methods, a system was evolved by which the methods in question are subjected to the most rigorous and painstaking scrutiny before they can be adopted. A "referee" is appointed for any subject for which the Association has not

yet an official method or for a method which seems to require further investigation. The referee conducts analyses according to the methods suggested for adoption in comparison with methods already established, obtaining the collaboration of as many as possible of the workers in that field. In addition, a great deal of original research has been inaugurated on new methods. This system developed logically until at the present time, in order to be adopted as "tentative," a method must be recommended to the Association by the referee, and such recommendation is made only after the method has undergone a thorough collaborative and critical study. Further, the special committee on methods must approve the recommendation and the method must be accepted by a vote of the Association. In order to become "official," a method must be again accepted at another annual meeting. The recommendations of referees are published in the reports of the proceedings of the Association in the *Journal of the Association of Official Agricultural Chemists*, so that all tentative methods are made public before being adopted. This permits consideration and criticism by chemists who are not members of the Association. It is immediately apparent that a method can be made official only after the most thorough series of tests, not alone for accuracy, but for ease of operation as well. It may be stated without reservation that more elaborate and painstaking effort has been expended on this collection of analytical methods than upon any other set of similar methods in the field of chemical science.

The compilation and revision of the methods presented in this book was made by a committee of the Association, consisting of R. E. Doolittle (chairman), B. L. Hartwell, G. W. Hoover, A. F. Seeker (deceased), J. P. Street, and W. A. Withers. Later, on the resignation of J. P. Street, A. J. Patten was appointed a member of the committee and the work of revision was continued.

A preliminary revision, antedating the revision published in this book, was printed in 1916 as supplementary parts to Volumes I and II of the *Journal of the Association of Official Agricultural Chemists*. In this preliminary revision the committee received important assistance from R. L. Emerson, F. C. Blanck, and N. A. Parkinson. At that time the scheme of numbering the sections in each chapter was adopted in order to simplify the system of cross-references.

In the preparation of the present revision J. A. MacLaughlin rendered valuable assistance. Acknowledgment is also made to the Library of the Department of Agriculture for assistance.

Throughout its work, it has been the aim of the committee not only to bring the methods up to date, but especially to state the procedure with such lucidity and in such detail as to make it possible for any trained chemist to operate without being in doubt at any time.

The work of the committee has been one of critical revision, compilation and editing. The work of developing the methods was done by the various referees and their collaborators who have reported to the Association at its annual meetings during the last decade. To them is due the credit for the subject matter of this book.

C. L. ALSBERG  
*Secretary of the Association of  
Official Agricultural Chemists*

September 17, 1920

## INTRODUCTION TO FIRST EDITION

---

By DR. HARVEY W. WILEY, Honorary President of the Association of  
Official Agricultural Chemists

In the present edition of the methods of analysis, official and tentative, of the Association of Official Agricultural Chemists, the technic of analytical procedures has been revised to November 1, 1919. The monumental work of the Association of Official Agricultural Chemists is not only well known in the profession in this country, but is recognized in all countries as being the last word in agricultural chemical technic. The methods of determining the composition of agricultural products, as well as of all bodies related to agriculture, has been recognized also by the courts of this country. In case of judicial proceedings where different methods of analysis have been employed, the court, in all cases where the question has arisen, has recognized the official methods as binding.

At the time of the organization of this body, referred to in the Preface, agricultural methods of research, from the chemical point of view, were extremely chaotic. The progress of agricultural science which has marked its history in the last third of a century could not have been maintained amid these chaotic conditions. The methods adopted by the founders of this Association for correcting this state of affairs have been shown by experience to be the best possible. I can say that the improvement in agricultural chemical technic has almost kept pace with the growth of the Association.

The gradual incorporation in the membership of the Association of those scientific men engaged in the control of foods and drugs has widened the scope without altering the purpose of the original founders. Today we find a body of scientific workers in agriculture and related subjects numbering quite half a thousand, who, by their activities and collabora-

tion, have contributed to the pages of this volume, directly or indirectly. The scientific knowledge of agriculture which has been verified and extended by this association now forms the foundation of all agricultural improvement.

The profession of agriculture is the fundamental industry of this country. Everything which strengthens the foundations of this industry benefits the country at large. Our workers are not banded together for personal preferment, either in wages or in authority. They have united for the sole purpose of benefitting agriculture and thus increasing production. They have not asked for shorter hours, nor for higher pay. They have worked in season, out of season, by day and by night, on work days and holidays, to perfect that science which, in its application, is the most powerful factor in scientific agriculture.

The ability of the agricultural industry to withstand the assaults which are made upon it at the present time is largely due to the successful efforts of our Association. The agricultural industry has been built upon a rock and thus it is able to withstand the winds and the floods. This industry is now in a more critical condition than any other. The allurements of the city, and the high wages of labor therein, have drawn from the farm much of its best blood and energy. Congregate life has become so much more attractive than discrete life that it is hard to keep the young of both sexes upon the farm. Yet it is plain that if man power and woman power upon the farm now be depleted the industry must suffer. Making the farm attractive does not merely mean beautifying the house in which the farmer lives, making it more sanitary, planting trees, flowers and shrubs, but it means also the best knowledge of the soil and its properties; the most scientific data respecting the manufacture and use of fertilizing materials; the most accurate knowledge of the character of crops best suited to the soil, and the best system of rotation which will help develop from the soil its most generous contribution. In other words, not only must the farmer's farm be attractive and sanitary, but it must also be productive and dividend paying.

We can well imagine the worth of the work which our Association has done by picturing for a moment what the present agricultural industry would be if all that our science has contributed to it were stricken from human records. In such a deplorable condition starvation would surely be staring the world in the face. In the quiet corners of the laboratory, by the midnight oil and by personal devotion, the means which enable the farmer to get more remunerative crops have been worked out and perfected. These workers, male and female, who have done this gigantic task have never been heralded in the public press, nor received encomiums of an admiring world. They have done their work silently and effectively, without expectation of praise and without hope of pecuniary reward.



Their real reward has been in the consciousness of duty done. The referees who have presided over this great work for the past thirty-six years and those who have aided them in these investigations, merit the generous regard and esteem of the whole scientific world, as well as the whole agricultural world. Our Association has been not one of debate nor of visionary plans of human welfare, but rather of hard work and concentrated devotion to the cause.

The volume which is now laid before you contains the very last word of all that is important in agricultural research from the chemical and physical point of view. This does not mean that the field is fully exploited. The great unknown of tomorrow doubtless holds in its secret embrace even greater prospects for human betterment than the days which have already passed. This Association stands ready and with expectant breath to receive the messages of tomorrow and translate them to the agricultural world.

Washington, D. C.,  
September 15, 1920

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\* The subject matter of the following chapters: III, Sewage; V, Agricultural Dust; XXIV, Fish and Other Marine Products; XXXVI, Vitamins; XL, Bacteriological Methods; and XLI, Microchemical Methods, indicate activities which the Association has begun or is planning to undertake.

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## DEFINITIONS OF TERMS AND EXPLANATORY NOTES

---

- (1) The term "water" used in the methods means distilled water.
- (2) The reagents listed below, unless otherwise specified, have the approximate strength stated and conform in purity with the requirements of the United States Pharmacopoeia.

Sulfuric acid . . . . .	Specific gravity 1.84
Hydrochloric acid . . . . .	Specific gravity 1.184
Nitric acid . . . . .	Specific gravity 1.42
Fuming nitric acid . . . . .	Specific gravity 1.50
Glacial acetic acid . . . . .	Specific gravity 1.048 (25°)
Hydrobromic acid . . . . .	Specific gravity 1.38
Phosphoric acid . . . . .	85 per cent strength by weight
Ammonium hydroxide . . . . .	Specific gravity 0.90
- (3) All other reagents and test solutions, unless otherwise described in the text, conform to the requirements of the United States Pharmacopoeia or of the American Chemical Society. When the anhydrous salt is intended, it is so stated; otherwise the salt referred to is the crystallized product.
- (4) In the expressions (1+2), (5+4), etc., used in connection with the name of a reagent, the first numeral indicates the volume of the reagent used, and the second numeral indicates the volume of water. For example, hydrochloric acid (1+2) means a reagent prepared by mixing one volume of hydrochloric acid with two volumes of water. When one of the reagents is a solid the expression means parts by weight, the first numeral representing the solid reagent and the second numeral the water.
- (5) In making up solutions of definite percentage it is understood that  $x$  grams of substance is dissolved in water and made up to 100 cc. Although not theoretically correct, this procedure will not result in any appreciable error in any of the methods given in this book.
- (6) For the sake of simplicity the abbreviations Cl and I instead of Cl<sub>2</sub> and I<sub>2</sub> are used for chlorine and iodine. Similar abbreviations have been used in other cases.
- (7) All calculations are based on the table of international atomic weights, Table I, under XLII.
- (8) Unless otherwise indicated all temperatures are expressed as degrees Centigrade.
- (9) Directions for standardizing reagents are given in Appendix 1.



# Official and Tentative Methods of Analysis

OF THE

## ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS

### I. SOILS<sup>1</sup>—TENTATIVE

#### 1 DIRECTIONS FOR SAMPLING

(In view of the variability in soils, it seems impossible to devise an entirely satisfactory method for sampling. It is obvious that the details of procedure should be determined by the purpose for which the sample is taken.)

Remove from the surface all vegetable material not incorporated with the soil. Take a sufficient number of samples to insure a composite sample that will be representative of the tract sampled, to the average depth of plowed soil, usually about 7 inches, and also take a composite sample from each important and distinctly different soil stratum to the depth of 40 inches, using a soil tube or auger. In using a soil auger, enlarge the first boring before boring below the plowed depth and carefully clean out the hole to prevent contamination of the successive sub-strata while withdrawing the samples. Do the sampling when the soil is reasonably dry. Thoroughly mix the samples of each depth and dry them in a well ventilated, cool place.

To calculate the percentage results obtained by analysis to pounds per given area of the soil, determine the weight of a given volume of the soil as it lies in the field.

#### 2 PREPARATION OF SAMPLE

(a) Reduce any soil lumps in the air-dried soil by rubbing in a porcelain mortar or by any other equally effective method that will not reduce the rock fragments, and pass thru a sieve having circular openings 1 mm in diameter. Thoroughly mix the sifted material and preserve in a suitable stoppered container. Weigh, and discard the detritus.

(b) If necessary for the determination of the total quantity of any constituent, pulverize more finely a sub-sample of (a).

Record any deviations from this procedure that are deemed necessary.

#### 3 MOISTURE

Dry to constant weight, in a wide-mouthed weighing bottle at 100°, 2 g of the prepared sample, 2(a). Report the loss in weight as percentage of the moisture-free weight of the sample taken.

#### 4 LOSS ON IGNITION

(This method gives only an approximation of the organic matter content, especially for soils containing much combined water.)

Ignite the soil from 3 to full redness in a Pt dish or suitable substitute, stirring occasionally, until organic matter is destroyed. If the soil contains appreciable quantities of carbonates, moisten, after cooling, with a few drops of a saturated soln

of  $(\text{NH}_4)_2\text{CO}_3$ , dry, heat to  $200^\circ$  for 30 min. to expel the  $\text{NH}_4$  salts, cool in a desiccator, and weigh. Report the percentage loss in weight as organic matter.

#### CARBONATE CARBON

5

#### APPARATUS

*Quadruplicate shaking apparatus for evolution of carbonate carbon in soils of high or low carbon content.*—This apparatus (Figs. 1 and 2) consists of a horizontal holder (H) 21 inches long,  $\frac{7}{8}$  inch thick, and  $1\frac{1}{4}$  inches wide, having properly spaced slots

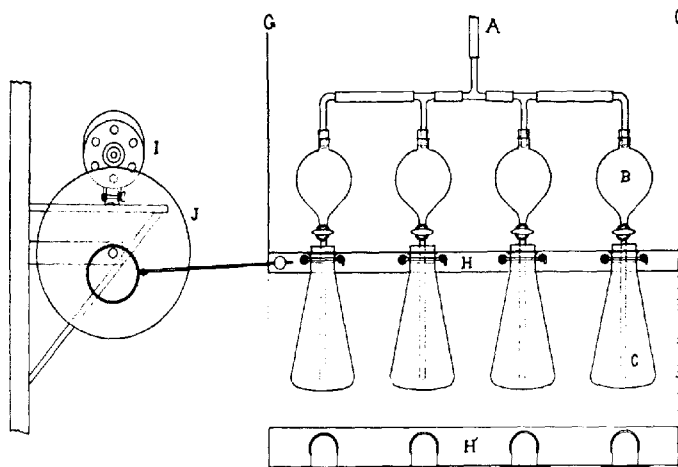


FIG. 1.—QUADRUPLICATE SHAKING APPARATUS FOR DETERMINATION OF CARBONATE CARBON IN SOILS

made to fit loosely the neck of a 300 cc Erlenmeyer flask taking a No. 6 rubber stopper. The holder is suspended horizontally from a bar by means of brass strips  $1\frac{1}{4}$  inches wide and 24 inches long. The common intake for purification of the incoming air leads from a tube about 25 inches long.

This tube stands upright, extending thru a rubber stopper in a 1 liter Erlenmeyer flask and, to prevent the mechanical carrying over of any of the purifying  $\text{NaOH}$ , it has inserted in the top a large N distillation bulb.

The driving wheel (J) is  $\frac{1}{2}$  inch thick and 7 inches in diameter. The eccentric attached to its face is  $\frac{1}{2}$  inch in thickness and 2 inches in diameter, and is grooved to permit free rotation of the driving shaft, which is fastened to the end of the holder by means of a binding post. Power for agitation is supplied by the motor (I), a sewing machine, or small desk fan motor. If the motor available has no rheostat, its speed can be easily controlled by a battery of 4 lamps. The motor is hinged upright on the support so that the pulley will rest upon the edge of the driving wheel. To reduce noise the pulley of the motor is inserted into a rubber stopper. Or the driving wheel may be made to carry a belt that is driven by the pulley of a small motor.

The absorber tower (D) is at least 25 inches high and 1 inch in diameter. It contains alternating pockets of solid glass rods and small glass beads resting upon an inverted test tube  $2\frac{1}{4}$  inches long. The rubber connection on the intake cock of

the tower is used to disconnect the glass tube that extends to the rubber connection on the safety bulb tube leading from flask C.

## 6

## DETERMINATION

*I. Volumetric Method<sup>2</sup>*

Pulverize the sample to pass a 60-mesh sieve, so as to expose fully to the action of the liberating acid any calcite that may be included in quartz crystals. For soils low in carbonates use a 10-, 25-, or 50-g charge in the quadruplicate shaking device described under 5.

Introduce the charge into the 300 cc evolution flask (C, Figs. 1 and 2), and aspirate 5 min. to free the apparatus of atmospheric  $\text{CO}_2$ ; release the suction, and introduce 10, 25, or 50 cc of 0.5 N NaOH or KOH soln into the absorption tower. Apply a suction of 5 inches and introduce 60 cc of HCl (1+9) containing 5% of  $\text{SnCl}_2$  upon the soil contained in the Erlenmeyer flask, regulating the intake of air by means of a screw cock placed just beyond the absorption tower. Agitate and aspirate for 60 min. at the rate of 3-4 bubbles per second. Then release the suction and draw off the absorbent soln into a 500 cc flask, washing the tower with a succession of fillings of  $\text{CO}_2$ -free  $\text{H}_2\text{O}$  to a volume of 450 cc. Add 10 cc of neutral aqueous soln of  $\text{BaCl}_2$  (250 g of  $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$  per liter), make to volume, agitate, and allow to stand 4 hours. Titrate the excess of hydroxide, using phenolphthalein indicator. With a small-bore buret permitting split-drop readings to hundredths of a cc, use 0.5 N acid for the titration; with burets of larger bore, use 0.1 or 0.25 N acid. Calculate and report the result as percentage of carbonate C or  $\text{CO}_2$ .

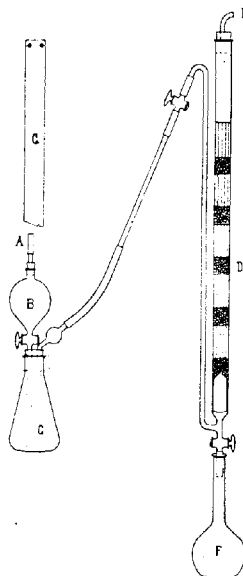


FIG. 2.—ABSORPTION TOWER

## 7

*II. Gravimetric Method<sup>3</sup>*

Proceed as in 6, but in lieu of the NaOH and KOH absorbent use an absorption tube filled with ascarite and preceded in the train by tubes containing an  $\text{Ag}_2\text{SO}_4$  suspension in  $\text{H}_2\text{SO}_4$ (1+19),  $\text{H}_2\text{SO}_4$ , and  $\text{CaCl}_2$  in order. Report the increase in the weight found for the ascarite tube in percentage of carbonate C or  $\text{CO}_2$ .

NOTE.—Special consideration should be given to the soils that have been treated with magnesite or dolomite or those known to be derived from the limited magnesite area, or from the glaciated region where transported dolomite may occur in considerable quantities. For such soils, agitate the HCl- $\text{SnCl}_2$ -soil suspension until  $\text{CO}_2$  evolution has subsided. Then apply heat to the agitated suspension until no  $\text{CO}_2$  evolution is indicated by the liquids in the absorption train. Remove heat, discontinue agitation, and draw  $\text{CO}_2$ -free air thru the apparatus for 20 min. The absorption may be accomplished by either the volumetric (6) or the gravimetric (7) procedure.



## ORGANIC CARBON

I. Furnace Combustion Method<sup>1</sup>

8

## APPARATUS

(a) *Oxygen cylinder*.—With pressure-regulating valve.

(b) *Electric combustion furnace*.—With rheostat, and with  $\frac{3}{4}$  × 24-inch fused silica tube containing an 8-inch loosely packed core of platinized asbestos. (CuO may be used if a temp. of 950° is not exceeded.)

(c) *Purification and absorption train*.—Place 2 scrubber bottles containing a 10% soln of KOH, followed by an Hg valve, between the O supply and the intake end of the furnace. Provide an asbestos-filled Cu coil with handle as an insulating plug on the intake end of the combustion tube; also use an asbestos plug to insulate the rubber stopper at the outlet end of the combustion tube. The outcoming current is dried and purified by an H<sub>2</sub>SO<sub>4</sub> scrubber, a tube containing 40-mesh granulated Zn, and a tube of P<sub>2</sub>O<sub>5</sub>, or equivalent drying material, in order. Connect the drying tube with a Nesbit or similar absorption tube filled with alternate layers of glass wool and ascarite and protect against moisture and back pressure at its outlet by a Fisher bubble counter containing H<sub>2</sub>SO<sub>4</sub>.

9

## DETERMINATION

Bring the furnace to a temp. of 900–950°. Connect the train and sweep out the apparatus by an adjusted flow of O. Weigh the absorption tube against a counterpoise, replace it in the train, and introduce well within the heated zone an alundum boat containing a 2 g charge of soil admixed with 2 g of finely divided CuO. Close the intake and open the Nesbit bulb. When no more gas passes thru the absorption train, connect it with suction, admit a flow of O, and aspirate for 30 min. Close the Nesbit tube, disconnect, and weigh against the counterpoise. Correct the total evolution of CO<sub>2</sub> for the carbonate CO<sub>2</sub> (determined as directed under 6 or 7) and report as organic C, or CO<sub>2</sub>. (There should be no carbonates in the residue in the case of acid, calcareous, or dolomitic soils.) Correct unleached alkali soils for the original and any residual CO<sub>2</sub>.

## II. Wet Oxidation Method

10

## REAGENTS

(a) *Oxidizing soln*.—Dissolve 85 g of CrO<sub>3</sub> in 100 cc of H<sub>2</sub>O and make to 250 cc with 85% H<sub>3</sub>PO<sub>4</sub>.

(b) *Acid soln*.—Mix equal volumes of 85% H<sub>3</sub>PO<sub>4</sub> and boiled H<sub>2</sub>SO<sub>4</sub>.

11

## DETERMINATION

Introduce 1–5 g of soil, depending upon the organic matter content, into each of the four 300 cc Pyrex Erlenmeyer flasks, 5. In addition, insert a glass bulb of about 1½ inches diameter between the Erlenmeyer flasks and the absorption towers and bend the tube leading from this bulb into the Erlenmeyer flask so as to permit the return of the condensed H<sub>2</sub>O along the side of the flask. In order to guard against the mechanical aspiration of the liberating acids, place an empty absorbent bead-filled tower or preferably a small condenser between each glass bulb and the tower that contains the hydroxide absorbent. Free the apparatus of atmospheric CO<sub>2</sub> and then introduce into each absorption tower 25 or 50 cc of 0.5 N NaOH or KOH. Apply suction to obtain 5 inches of vacuum and run into each Erlenmeyer flask 10 cc of

the oxidizing soln. Add 25–40 cc of the acid mixture. Gently agitate the flasks and place a low flame under each. Continue the gentle agitation and heating for 30 min. after the mixture begins to boil.

At the end of the agitation and aspiration, release the suction and wash the absorbent into a 500 cc flask. Add 10 cc of a neutral aqueous soln of  $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$  (250 g of  $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$  per liter). Dilute to volume and permit the precipitate of  $\text{BaCO}_3$  to settle at least 4 hours (preferably overnight). Pipet an aliquot of 200 cc and titrate the residual hydroxide with 0.5 *N* acid, using phenolphthalein indicator and splitting drops by the use of a stirring rod near the end of the titration.

Residual hydroxide in terms of 0.5 *N* alkali minus the 0.5 *N*  $\text{NaOH}$  originally used = the  $\text{CO}_2$  present in the sample. Total  $\text{CO}_2$ , corrected for carbonate  $\text{CO}_2$  as determined under 6, = percentage of  $\text{CO}_2$  derived from the oxidation of the organic material.

#### TOTAL NITROGEN

12

##### *Gunning-Hibbard Method*

Digest 10 g of soil in a 500 cc Kjeldahl boiling flask with 30–40 cc of  $\text{H}_2\text{SO}_4$  and approximately 10 g of salt mixture composed of 10 parts of  $\text{K}_2\text{SO}_4$  or anhydrous  $\text{Na}_2\text{SO}_4$ , 1 part of  $\text{FeSO}_4$ , and  $\frac{1}{2}$  part of  $\text{CuSO}_4$ . Continue the digestion until the mixture is colorless or nearly so. After cooling, dilute the contents of the flask with  $\text{H}_2\text{O}$ , add an excess of an approximately 45%  $\text{NaOH}$  soln, connect the flask with the condenser, and distil 150 cc into standard acid as directed under II, 21. (The distillation may be carried out in the digestion flask, or, if preferred, the soln may be transferred to an Armsby Cu pot.) Titrate the excess of acid with 0.1 *N* or *N*/14 alkali, using methyl red or cochineal indicator, II, 19(h) and (i). Report as percentage of N.

13

##### *Kjeldahl Method*

Proceed as directed under 12, using 0.7 g of  $\text{HgO}$  or 0.65 g of  $\text{Hg}$  instead of the salt mixture. Mix immediately and heat over a low flame, gradually increasing the heat. Continue the digestion until the mixture is colorless or nearly so. After cooling, dilute the contents of the flask; add 25 cc of sulfide or thiosulfate soln, II, 19(f), and an excess of the  $\text{NaOH}$  soln; and proceed with the distillation and titration as directed under II, 21. Report as percentage of N.

#### SODIUM CARBONATE FUSION<sup>6</sup>

14

##### *Method I*

Thoroughly mix on glazed paper 3 g of soil, ground to 100-mesh, with 15 g of  $\text{Na}_2\text{CO}_3$ , and transfer the mixture carefully to a 100 cc Pt crucible. Cover the crucible, heat at low redness until fusion begins, then increase the heat until a clear, quiet fusion results; finally, give full heat of a Meker burner for 20 min., having the flame oblique to insure good oxidation. Pour the fusion into a large Pt dish set in  $\text{H}_2\text{O}$ . Place the crucible and cover in a wide 400 cc beaker and cover with  $\text{H}_2\text{O}$ . Transfer the fused lump from the Pt dish to the beaker, and rinse the dish into the beaker with  $\text{HCl}$  (1+9). Add 50 cc of  $\text{HCl}$  to the contents of the beaker, cover, and keep on a steam bath until the fused mass has disintegrated. Transfer the mixture to a 250 cc porcelain or quartz dish and evaporate to dryness on a steam bath.

15

##### *Method II*

Proceed as directed under 27.

## 16

## SILICA

Take up the residue from 14 or 15 in HCl (1+9) and filter the mixture so obtained (a 9 cm Büchner funnel with suction may be used advantageously). Wash with hot H<sub>2</sub>O containing 5 cc of HCl per liter. Collect the filtrate and washings in a dish, preferably a casserole, and dehydrate on a steam bath until the SiO<sub>2</sub> assumes a crystalline appearance. Moisten with HCl and repeat the dehydration for 2 hours. Add 5 cc of HCl and 100 cc of hot H<sub>2</sub>O, mix thoroly, filter, and wash. Add the residue to the main portion of SiO<sub>2</sub> obtained from the first filtration. Make up the combined filtrate and washings to 500 cc at 20° and save for subsequent determinations. Place the two SiO<sub>2</sub> residues with filters in a porcelain crucible. Moisten with a saturated NH<sub>4</sub>NO<sub>3</sub> soln. Ignite with low heat at first to burn off filter paper and then with a strong flame, preferably a blast lamp, to constant weight; cool in a desiccator and weigh. Report as percentage of SiO<sub>2</sub>.

## 17 OXIDES OF IRON, ALUMINUM, MANGANESE, PHOSPHORUS AND TITANIUM

To a 100 or 200 cc aliquot of the soln from 16, according to the probable quantity of Fe and Ca present, add NH<sub>4</sub>OH (1+1) dropwise until the precipitate formed requires several seconds to dissolve, thus leaving the soln faintly acid. Add 0.5 g of solid NH<sub>4</sub> persulfate, heat nearly to the boiling point, and add sufficient NH<sub>4</sub>OH (1+1) to precipitate all Fe, Al, etc. Allow the mixture to boil in a covered beaker for about 1 min., remove, and if no NH<sub>3</sub> is given off (detected by smelling), again add NH<sub>4</sub>OH dropwise until it can be detected. Do not allow the precipitate to settle, but stir and pour on the filter. Wash immediately with hot 2.5% NH<sub>4</sub>NO<sub>3</sub> soln, playing a fine jet around the edge of the precipitate, thus cutting it free from the paper in order to insure rapid filtration. Wash the precipitate several times. Transfer the paper and the precipitate to the original beaker, add 5 cc of HCl, macerate quickly, add 50 cc of H<sub>2</sub>O, and heat. (This procedure insures that the precipitate will not be carried to the gel state.) Reprecipitate the oxides with NH<sub>4</sub> persulfate and NH<sub>4</sub>OH (1+1) as directed above, filter, and wash with hot 2.5% NH<sub>4</sub>NO<sub>3</sub> soln until free from chlorides. Reserve the filtrate and washings from both the first and second precipitations for the determination of Ca and Mg.

Dry the precipitate, remove from the filter, and ignite in a Pt crucible over a Bunsen flame, incinerating the filter separately, and add the residue to the precipitate. Then ignite to bright redness, cool in a desiccator, and weigh as Fe<sub>2</sub>O<sub>3</sub>, Al<sub>2</sub>O<sub>3</sub>, Mn<sub>2</sub>O<sub>4</sub>, TiO<sub>2</sub>, and P<sub>2</sub>O<sub>5</sub>.

To this residue add KHSO<sub>4</sub> or K<sub>2</sub>S<sub>2</sub>O<sub>7</sub>, and heat at low temp. until the precipitate is completely disintegrated; cool quickly and transfer to a flask containing 100 cc of H<sub>2</sub>SO<sub>4</sub> (1+3). Dissolve the melt, reduce with Zn, cool, and determine Fe by titration with 0.2 N KMnO<sub>4</sub> soln (XXXVII, 59). Report as percentage of Fe<sub>2</sub>O<sub>3</sub>.

Or, in lieu of the above fusion, evaporate 50 or 100 cc of the soln from 16 after the addition of 10 cc of H<sub>2</sub>SO<sub>4</sub> until all HCl is expelled. Dilute with H<sub>2</sub>O, reduce with Zn, and determine the Fe by titration with 0.2 N KMnO<sub>4</sub> soln.

Evaporate 50–100 cc of the soln from 16 after the addition of 10 cc of HNO<sub>3</sub>. Repeat the addition of HNO<sub>3</sub> and evaporation to insure expulsion of all HCl. From this point proceed as directed under XXXVII, 75. Subtract the sum of the oxides of Fe, Mn, and P (determined separately as directed in 29 or 30) from the weight of the combined oxides of Fe, Al, Mn, P, and Ti, determined as directed above. Report the remainder as oxides of Al and Ti.

## 18

## CALCIUM

Concentrate the combined filtrates and washings from 17 to about 50 cc; make slightly alkaline with NH<sub>4</sub>OH (1+1); and add, while still hot, saturated NH<sub>4</sub>

oxalate soln dropwise as long as any precipitate is produced, and then an excess sufficient to convert the Mg salts also into oxalate. Heat to boiling, allow to stand 3 hours or longer, decant the clear soln thru a filter, pour 15–20 cc of hot  $\text{H}_2\text{O}$  on the precipitate, and again decant the clear soln thru the filter. Dissolve the precipitate in the beaker with a few drops of  $\text{HCl}$ ; add a little  $\text{H}_2\text{O}$ ; and reprecipitate, boiling hot, by adding  $\text{NH}_4\text{OH}$  and a little  $\text{NH}_4$  oxalate soln. Allow to stand as before and filter thru the same filter. Wash free from chlorides with hot  $\text{H}_2\text{O}$ . Reserve the filtrates and washings from both precipitations for the determination of Mg under 19 or 21. Complete the determination by one of the following procedures and report as percentage of  $\text{CaO}$ .

(a) Ignite the precipitate in a crucible either over an S-free blast or in an electric oven at  $950^\circ$  to constant weight, cool in a desiccator, and weigh the  $\text{CaO}$ .

(b) Incinerate the filter over a low flame, mix the ignited precipitate with a finely pulverized and dried mixture of equal parts of  $(\text{NH}_4)_2\text{SO}_4$  and  $\text{NH}_4\text{Cl}$ , and drive off the excess of the sulfate by careful heating of the upper portion of the crucible. Complete the ignition, cool in a desiccator, and weigh the  $\text{CaSO}_4$ .<sup>5</sup>

(c) Dissolve the  $\text{Ca}$  oxalate precipitate from the filter with hot  $\text{H}_2\text{SO}_4$  (1+1), collect the soln in the beaker employed for precipitation, and titrate while hot with 0.1 N  $\text{KMnO}_4$  (XII, 10). Then add the unmacerated filter paper to the soln and complete the titration.

#### MAGNESIUM

19

##### Method I

Evaporate the combined filtrates and washings from 18 on a water bath to about 100 cc and add cautiously 20–30 cc of  $\text{HNO}_3$ . Cover the beaker, evaporate to dryness on a hot plate to remove  $\text{NH}_4$  salts. Add 5 cc of  $\text{HCl}$  and evaporate nearly to dryness. Dissolve the residue in hot  $\text{H}_2\text{O}$  and a small quantity of  $\text{HCl}$ . If necessary, filter the soln and wash the filter paper with about 100 cc of hot  $\text{H}_2\text{O}$ . Precipitate the Mg as  $\text{MgNH}_4\text{PO}_4$  by the addition of 3 cc of a 10% soln of  $\text{NH}_4$  phosphate and sufficient  $\text{NH}_4\text{OH}$  to make the soln slightly alkaline. Stir the soln vigorously, allow to stand 15 min., add 15 cc of  $\text{NH}_4\text{OH}$ , and allow the precipitation to proceed overnight. Filter, wash the precipitate with  $\text{NH}_4\text{OH}$  (1+9), transfer to a porcelain crucible, moisten the filter with  $\text{NH}_4\text{NO}_3$ , dry, ignite, cool, and weigh as  $\text{Mg}_2\text{P}_2\text{O}_7$ . The filtration may be made thru a Gooch crucible. Calculate and report the result as percentage of  $\text{MgO}$ .

##### Method II<sup>7</sup>

20

##### REAGENT

*Phosphate soln.*—Dissolve 100 g of  $(\text{NH}_4)_2\text{HPO}_4$  or  $\text{NaNH}_4\text{HPO}_4 \cdot 4\text{H}_2\text{O}$  in hot  $\text{H}_2\text{O}$ , cool, and dilute to 1 liter.

21

##### DETERMINATION

To the combined filtrates and washings from 18, add 100 cc of  $\text{NH}_4\text{OH}$  and 50 cc of 95% alcohol. Then add with constant stirring 25 cc of the phosphate soln and let stand 12–24 hours. Filter, wash twice with  $\text{NH}_4\text{OH}$  (1+9) and dissolve the precipitate in  $\text{HNO}_3$  (1+4), washing the soln into the original beaker to a volume of 100–150 cc. To this soln add  $\frac{1}{10}$  volume of  $\text{NH}_4\text{OH}$  and 2 drops of the phosphate soln. Stir vigorously and allow to stand 3 hours or longer. Filter thru a Gooch crucible, wash with the  $\text{NH}_4\text{OH}$ , moisten the filter with saturated ammoniacal soln of  $\text{NH}_4\text{NO}_3$ , ignite, and weigh as  $\text{Mg}_2\text{P}_2\text{O}_7$ . Calculate and report the result as percentage of  $\text{MgO}$ .

## MANGANESE

22

*Method I*

Treat 1 g of 100-mesh soil with 5 cc of HF and 5 cc of  $\text{H}_2\text{SO}_4$  (1+1). Evaporate to dryness, ignite, and fuse the residue with  $\text{KHSO}_4$ . Repeat the addition of HF until all silicates are decomposed. Dissolve in  $\text{H}_2\text{O}$ , add  $\text{HNO}_3$ , and evaporate to dryness. Again dissolve in  $\text{H}_2\text{O}$ , add 25 cc of  $\text{HNO}_3$  (1+2) and about 0.5 g of Na bismuthate, and heat until the permanganate color disappears. From this point proceed as directed under XXXVII, 75, beginning with "Add a few drops of a 10% soln of  $\text{NH}_4$  bisulfite or saturated Na bisulfite to clear the soln." Report as percentage of mangano-manganic oxide ( $\text{Mn}_3\text{O}_4$ ).

*Method II<sup>s</sup>*

23

## REAGENT

*Standard manganous sulfate soln.*—Dissolve 0.2877 g of pure  $\text{KMnO}_4$  in about 100 cc. of  $\text{H}_2\text{O}$ , acidify the soln with  $\text{H}_2\text{SO}_4$  (1+1), and slowly heat to boiling. Add slowly a sufficient quantity of a 10% oxalic acid soln to discharge the color. Cool, and dilute to 1 liter. 1 cc = 0.1 mg of Mn.

24

## DETERMINATION

Weigh 0.5–5.0 g of finely pulverized, air-dried soil into a 50 cc quartz or Pt crucible. Add to the soil approximately  $2\frac{1}{2}$  times its weight of finely powdered, Mn-free  $\text{KHSO}_4$ , and mix thoroly. Place the lid on the crucible and heat gently over a Bunsen burner about 5 min.; increase the heat gradually until the crucible and lid are red hot, being careful not to allow the contents of the crucible to froth over. Continue to heat for about 20 min., or until the frothing has ceased and the contents are in a quiet molten condition. Withdraw the flame from beneath the crucible, remove the lid, and rotate the crucible in a horizontal position to spread the molten contents over the inner walls to expedite cooling. When the crucible is no longer red, immerse it in about 25 cc of  $\text{H}_2\text{SO}_4$  (1+1) in a 250 cc beaker and digest on a hot water bath until the contents of the crucible disintegrate and dissolve. Carefully rinse the crucible and lid with hot  $\text{H}_2\text{O}$  and dilute the soln to about 100 cc. Filter, and wash the insoluble residue.

Discard the insoluble residue if it has a uniform white color; if it is colored by undecomposed particles of minerals, ignite and expel the  $\text{SiO}_2$  with HF and  $\text{H}_2\text{SO}_4$ . Fuse the residue with  $\text{KHSO}_4$ , digest in  $\text{H}_2\text{SO}_4$  (1+1), and add the soln to the filtrate from the fusion.

Make the soln to a definite volume, take an aliquot for the determination, and add about 0.05 g of K periodate to the aliquot. Boil the soln until the characteristic purplish permanganic acid color develops, heat on a hot water bath for an hour, and set aside to cool. If the color is deep purple, dilute the soln to a definite volume. Remove an aliquot and match against a standard Mn soln in Nessler jars, or in a colorimeter. Compute the results as percentage of Mn or  $\text{Mn}_3\text{O}_4$ . (A series of Mn standard solns is prepared from the standard manganous sulfate soln by removing aliquots and developing the Mn color with K periodate in the same way as the soln of the sample.)

IODINE<sup>o</sup>

25

*Fusion Method*

Place 5 g of air-dry soil, ground to pass a 100-mesh sieve, 10 g of I-free KOH pellets and 5 cc of  $\text{H}_2\text{O}$  in a clean 400 cc iron crucible and stir with a clean piece of

No. 6 iron wire until most of the pellets have dissolved. Place the crucible in a 4.5 in. Bunsen tripod and heat moderately with the flame of a burner, stirring the contents of the crucible rapidly until the  $\text{H}_2\text{O}$  has been driven off and a dry granular fused mass remains. Avoid heating the crucible to redness after the  $\text{H}_2\text{O}$  has been expelled. Cool the crucible and add about 50 cc of  $\text{H}_2\text{O}$  and allow it to stand with occasional stirring until the fused mass has slaked to a sludge. Transfer the contents of the crucible to a 500 cc beaker, police, and wash the inside walls of the crucible thoroly. Add a small strip of litmus paper, about 0.1 g of K bisulfite, and  $\text{HCl}$  (1 + 1), stirring until the contents of the beaker have an acid reaction and a distinct odor of  $\text{SO}_2$  can be detected. Add a saturated soln of  $\text{K}_2\text{CO}_3$  from a short stem pipet, stirring

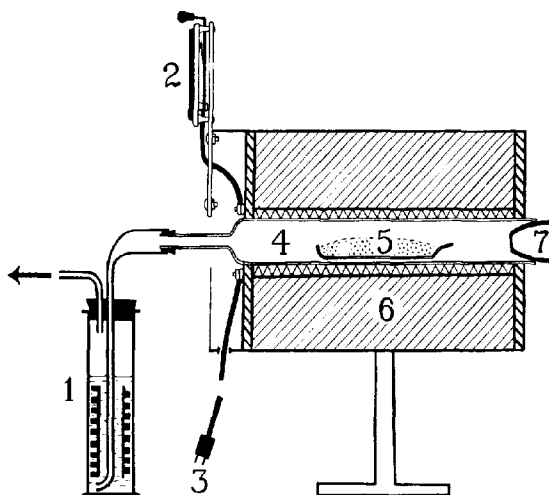


FIG. 3.—FURNACE USED IN DETERMINATION OF IODINE IN SOILS

1—Gas wash bottle, 2—Rheostat, 3—Power line, 220 volts, 4—Quartz tube, 5—Sample (in sillimanite boat), 6—Electric tube furnace, 7—Stopper (alundum crucible).

until the entire mass has an alkaline reaction. Pour the precipitate of silica, Fe and Al hydroxides onto a folded filter and wash thoroly by the addition of about 25 cc portions of hot  $\text{H}_2\text{O}$  at a time, allowing each portion to drain thru before adding another. (The volume of the filtrate and washings should be approximately 500 cc.) Transfer the filtrate to a porcelain dish and evaporate until a sludge of salts remains. (A small current of compressed air directed on the surface of the soln during heating on the water bath will hasten the evaporation.) Remove the dish from the water bath and add 50 cc of pure 95% ethyl alcohol to the hot sludge of salts. Stir thoroly with a policeman until the dish has attained room temp. (The salts assume a pasty consistency with much stirring while they are hot and this condition facilitates the soln of the KI in the alcohol.) Decant the alcoholic extract thru a small folded filter into a beaker, further extract the residue with one 25 and one 10 cc portion of alcohol, and decant thru the filter into the beaker containing the first alcoholic extract. Dissolve the salts adhering to the filter paper and those in the dish in hot  $\text{H}_2\text{O}$ , and

evaporate the soln to a sludge. Extract the sludge with one 25 cc and two 10 cc portions of alcohol as directed in the treatment of the first sludge of salts, and repeat the process of extraction, using three 10 cc portions of alcohol. Combine the alcoholic extracts and evaporate to dryness. Dissolve the residue in a small amount of hot  $H_2O$ , rinse into a 250 cc beaker, and evaporate to dryness. Dissolve the residue in a few drops of hot  $H_2O$ , a drop of a saturated soln of  $K_2CO_3$ , and add 10 cc of alcohol. Stir the precipitated salts rapidly with a glass rod for about 10 min., and decant the alcoholic extract thru a small filter into a 150 cc beaker. Further stir the residue, extract with two 5 cc portions of alcohol, decant thru the filter into the beaker, rinsing the filter with a few cc of alcohol, and evaporate the extract to dryness. Dissolve the residue, consisting of about 0.05 g, in a few drops of hot  $H_2O$  and rinse into a 25 cc Pt or sillimanite dish, evaporate to dryness, and dry at  $100^\circ$  for 1 hour. Heat the dish at about  $400^\circ$  in an electric furnace having a pyrometer attachment, until the organic matter is burned or charred so that it will not give a turbid soln when  $H_2O$  is added. If a turbid soln is obtained, evaporate to dryness and burn again. After cooling the dish, dissolve the residue in a few drops of distilled  $H_2O$ , at room temp., filter the soln, and wash into a 30 cc separatory funnel. (The soln should be colorless, slightly alkaline, and have a volume of about 5 cc.) Make slightly acid with a few drops of  $H_2SO_4$  (1 + 1), add about 0.01 g of pure K bisulfite to the separatory funnel, stopper, and shake for a few seconds to reduce any iodate to iodide. Remove the stopper, and add 1 cc of pure  $CS_2$  and about 2 cc of a 10% soln of pure I-free K or  $NaNO_2$ . Stopper and shake the separatory vigorously for about 1 min. Place the separatory in a stand and allow the  $CS_2$  containing the I to collect and settle for about 5 min. If the  $CS_2$  has a light pink color it contains all the I; if it has a deep pink color, run it carefully into a centrifuge tube and further extract the soln in the separatory with 1 cc portions of  $CS_2$  until the last portion has only a slight pink color. Combine the  $CS_2$  extracts and centrifuge. Place a portion of the clear extract in a micro cup of a colorimeter and compare quickly with a freshly prepared I standard having nearly the same depth of color. Report results in p.p.m.

## 26 *Volatilization Method by Heating Soil in an Electric Tube Furnace*

Place in a porcelain boat 25-100 g of soil ground to pass a 2-mm sieve and insert the boat in the silica tube, Fig. 3. Use 2 wash bottles (Milligan), each containing 100 cc. of 2.5% soln of  $K_2CO_3$ . Connect the first wash bottle with the silica tube by means of a glass thimble made to fit a rubber gasket on the small end of the combustion tube. Connect the wash bottles closely with rubber tubing. Attach the last wash bottle to a suction pump which is regulated to draw the vapors at a moderate rate into the wash bottles during the time the soil is heated. (About 1 hour is required to attain the maximum temp. of the furnace, which is maintained for about 2 hours.) Disconnect the wash bottles, rinse the soln into a porcelain dish, and evaporate to dryness. Dissolve the residue in a few drops of hot  $H_2O$ , rinse into a 150 cc beaker, and evaporate until about 2 cc remains. Add to the beaker 10 cc of pure 95% ethyl alcohol, stir rapidly with a glass rod for about 10 min., and decant the extract thru a small filter into a 150 cc beaker. Extract the residue with two 5 cc portions of alcohol and decant thru the filter into the beaker. Evaporate the alcohol and wash the residue into a 25 cc sillimanite dish, evaporate to dryness, dry at  $100^\circ$ , and heat at about  $400^\circ$  for 10 min. in an electric furnace having a pyrometer attachment. Remove the dish from the furnace, cool, and dissolve the residue in a few drops of cold distilled  $H_2O$ . Filter, and wash into a 30 cc separatory. Liberate, absorb, and determine the I as directed in 25.

SULFUR<sup>13</sup>

27

## PREPARATION OF SOLUTION

Weigh 5–10 g of the soil, prepared as directed under 2(b) to pass a 0.5 mm sieve, into a 100 cc Ni crucible; add an equal weight of anhydrous  $\text{Na}_2\text{CO}_3$ ; and mix well with a stout Ni stirring rod of such length as to permit introduction into the furnace to be used in the fusion. Pipet carefully 4 cc of  $\text{H}_2\text{O}$  into each 10 g of soil; stir well to a stiff paste, adding more  $\text{H}_2\text{O}$  if necessary, a few drops at a time. Immediately add successive portions of about 1 g of S-free  $\text{Na}_2\text{O}_2$ , stirring well after each addition to obviate excessive frothing and overflow. Continue to add peroxide until the mixture becomes dry and granular, and then add, as a surface coating, enough to make the total peroxide addition 25 g per each 10 g of soil. Place the mixture in an electric furnace; maintain a temp. between  $400^\circ$  and  $500^\circ$  during the first half hour, then raise it rapidly to bright red heat (about  $900^\circ$ ); and continue the fusion at this temp. for about 10 min. Withdraw the crucible from the muffle, quickly manipulate so as to cause the melt to spread out in a thin sheet over the interior of the crucible, and cool rapidly by contact with some good conductor in a cool atmosphere. Place the chilled crucible sideways in a 600 cc beaker and cover with  $\text{H}_2\text{O}$ . Add about 5 cc of 95% alcohol to decompose the Na manganate. Cover the beaker with a watch-glass, place on a cold hot plate, and apply heat. Boil briskly until all the melt is disintegrated (30 min. is ordinarily sufficient). When the suspension has assumed a flesh-colored, flocculent appearance, with no glassy green lumps in the interior of the crucible, remove the crucible and rod from the beaker and wash any flaky particles back into the beaker with the aid of a rubber-tipped glass rod, rinsing several times with hot  $\text{H}_2\text{O}$ . (If small glassy particles still cling to the inside of the crucible, disintegrate by boiling  $\text{H}_2\text{O}$  over the hot plate or a small flame and add to the main portion.) Filter immediately by suction thru a 9 cm Büchner funnel into a liter beaker placed under a bell jar. When no more of the liquid can be drawn thru the filter, return the residue, together with the filter paper, to the original beaker, washing any adhering particles carefully from the funnel. Add about 1 g of  $\text{Na}_2\text{CO}_3$ , macerate with the stirring rod, add 75–100 cc of  $\text{H}_2\text{O}$ , and bring to a brisk boil while stirring vigorously. Again filter thru a Büchner funnel, using suction, until nearly dry, and wash with 20 cc portions of hot  $\text{H}_2\text{O}$  to a total volume of 500 or 700 cc for the 5 and 10 g charges, respectively.

28

## DETERMINATION

Add from a buret slowly and with stirring, sufficient HCl to neutralize the soln, using methyl red indicator, II, 19(i). Add 0.5 cc excess of the HCl and concentrate by heating to a volume of 400 cc. If a cloudiness should appear, it is imperative to remove the  $\text{SiO}_2$  by evaporation and dehydration on an electric hot plate. If the soln is perfectly clear, heat to boiling, add slowly 10 cc of a 5%  $\text{BaCl}_2$  soln, and allow to stand overnight. Filter on a dense filter paper, place paper in a Pt crucible, and ignite in an electric furnace. Cool in a desiccator, and weigh as  $\text{BaSO}_4$ . To insure against possible inclusion of  $\text{SiO}_2$ , add 2 drops of HF and 1 drop of  $\text{H}_2\text{SO}_4$  (1+1), heat carefully, reignite, and weigh. Report as percentage of S or  $\text{SO}_3$ .

## PHOSPHORUS

29

*Sodium Peroxide Method*

Place 10 g of  $\text{Na}_2\text{O}_2$  in either an iron or porcelain crucible and thoroly mix with it 5 g of the prepared soil, 2(b). If the soil has a low organic matter content, add a little starch to hasten the action. Heat the mixture carefully by applying the flame



of a Bunsen burner directly upon the surface of the charge and the sides of the crucible until reaction starts; then cover the crucible and keep at a low red heat for 30 min. Do not allow fusion to take place. By means of a large funnel and a stream of hot  $H_2O$  transfer the charge to a 500 cc volumetric flask, acidify with  $HCl$  and boil. Cool, and make up to the mark. (If the action has taken place properly, there should be no particles of undecomposed soil in the bottom of the flask.) Allow the  $SiO_2$  to settle and draw off 200 cc of the clear soln.

Or, in lieu of the above, oxidize 5–10 g of the material and disintegrate the melt, as directed under 27. Dissolve the residue with  $HNO_3$  (1+1), dilute to 500 cc, and withdraw a 200 cc aliquot.

Precipitate the Fe, Al, and P with  $NH_4OH$  (1+1); filter; wash several times with hot  $H_2O$ ; return the precipitate to the beaker; and dissolve it in hot  $HCl$  (1+4), pouring the acid upon the filter to dissolve any precipitate remaining. Evaporate the soln and washings to complete dryness on a water bath. Take up with  $HNO_3$  (1+4), heating if necessary, and filter to remove  $SiO_2$ . Evaporate the filtrate and washings to about 10 cc and add 2 cc of  $HNO_3$ . Neutralize the excess of acid with  $NH_4OH$  (1+1) and then add  $HNO_3$  until the soln becomes clear, avoiding an excess of the acid. Heat to 40–50° in a water bath, add 15 cc of molybdate soln, II, 7(a), and keep at this temp. for 1–2 hours. Let stand overnight, filter, and wash free from acid with cold  $H_2O$ . Transfer the filter to a beaker and dissolve in standard  $NaOH$  or  $KOH$  (1 cc = 0.5 mg of  $P_2O_5$ ). Titrate the excess of alkali with standard  $HNO_3$ , using phenolphthalein indicator. Or, after adding the 15 cc of molybdate soln, allow to stand 3 hours at a temp. not above 45°, filter on a small filter or on a Gooch crucible, and wash with 2.5%  $NH_4NO_3$  soln and then with cold  $H_2O$  until two fillings of the filter do not greatly diminish the color produced with phenolphthalein by 1 drop of standard alkali. Return the filter and precipitate to the same beaker used for precipitating the phosphomolybdate, dissolve the yellow precipitate in standard  $NaOH$  or  $KOH$  (1 cc = 0.5 mg of  $P_2O_5$ ), add a few drops of phenolphthalein indicator, II, 10(d), and titrate the excess of alkali with standard  $HNO_3$ . Report as percentage of  $P_2O_5$ .

## 30

*Magnesium Nitrate Method*

Place 5 g of soil, prepared as directed under 2(b), in a porcelain dish. Moisten with 5–7 cc of  $Mg(NO_3)_2$  soln, II, 7(e). Dry on a water bath and burn off the organic matter at low redness. Cool, and add 10 cc of  $H_2O$ , 10 cc of  $HCl$ , and 5 cc of  $HNO_3$ . Cover the dish and digest the contents for 2 hours on a water bath, stirring 2 or 3 times during the digestion. Dilute to 250 cc, mix well, and filter thru a dry folded filter, pouring back thru the filter until the filtrate is clear. Place an aliquot corresponding to 2 or 4 g of the soil, depending upon the quantity of P present, in a hard glass beaker or porcelain dish and evaporate to dryness on a water bath. Take up with  $HNO_3$  (1+4), again evaporate to dryness, and heat for 1 hour at 110–120°. Again take up with the dilute  $HNO_3$  and filter. Reduce the combined volume of filtrates and washings to 30–40 cc. Make alkaline with  $NH_4OH$  (1+1), and dissolve the precipitate with  $HNO_3$ , using a slight excess. Add gradually with vigorous agitation 15 cc of molybdate soln, II, 7(a). Keep the soln at 45° for an hour and then let stand overnight at room temp. Filter, and wash well with 2.5%  $NH_4NO_3$  soln and then with cold  $H_2O$ . Return filter and precipitate to the same flask and determine P volumetrically as directed under 29. Report as percentage of  $P_2O_5$ .

## 31

**POTASSIUM AND SODIUM,<sup>11</sup> OR POTASSIUM ONLY**

Triturate gently 0.5 or 1 g of the finely ground soil with 1 g of dry  $NH_4Cl$  in a smooth mortar, add 8 parts of  $CaCO_3$ , and mix intimately. Transfer the mixture to

a Pt crucible, rinsing the mortar with a little  $\text{CaCO}_3$ . Heat the crucible gradually until fumes of  $\text{NH}_4$  salts no longer appear and the lower  $\frac{2}{3}$  of the crucible is brought to a red heat. Maintain this temp. 40–60 min. The temp. should be sufficient to keep the  $\text{CaCl}_2$ , formed by the reaction of  $\text{NH}_4\text{Cl}$  with  $\text{CaCO}_3$ , in a state of fusion. (The mass does not become a melt because the fused  $\text{CaCl}_2$  is absorbed by the large quantity of  $\text{CaCO}_3$  present. If the silicate is fused by the application of too strong heat, disintegration of the mass at the end of the operation with  $\text{H}_2\text{O}$  cannot be effected. Moreover, too high a temp. causes volatilization of alkali chlorides. The mass contracts in volume during the ignition and is usually easily detached from the crucible.) Transfer the fused mass to a porcelain dish, slake with hot  $\text{H}_2\text{O}$ , and grind thoroly with an agate pestle. After washing 5 times by decantation with hot  $\text{H}_2\text{O}$ , transfer to a filter and wash well (300 cc of wash water is sufficient). To the filtrate add sufficient  $(\text{NH}_4)_2\text{CO}_3$  soln to precipitate any Ca present. Allow to settle, decant the supernatant liquid into a porcelain dish, and concentrate by evaporation, finally transferring the precipitate to the dish. When the volume is reduced to about 30 cc, add a little  $(\text{NH}_4)_2\text{CO}_3$  and  $\text{NH}_4\text{OH}$ , heat, filter into a porcelain dish, evaporate the filtrate to dryness on a water bath, and expel  $\text{NH}_4$  salts by ignition; or evaporate with 10 cc of  $\text{HNO}_3$ , followed by 2 evaporations with 10 cc each of  $\text{HCl}$ .

If K alone is to be determined, proceed from this point as directed under II, 44(a), beginning with "Dissolve the residue in hot  $\text{H}_2\text{O}$ ." Report as percentage of  $\text{K}_2\text{O}$ .

If both K and Na are to be determined, dissolve the residual alkali chlorides in 3–5 cc of  $\text{H}_2\text{O}$  (a little black or brown flocculent matter usually remains undissolved), warm, and filter thru a small filter into a weighed Pt dish. Evaporate to dryness on a water bath, carefully heat the residual alkali chlorides to incipient fusion, cool, and weigh as Na and K chlorides. Dissolve the combined chlorides in 30 cc of  $\text{H}_2\text{O}$ , add 1.5 cc of  $\text{PtCl}_4$  soln, II, 42(b), evaporate to a sirupy consistency, and add 15 cc of 2.25 *N* acidulated alcohol (prepared by passing  $\text{HCl}$  gas into a mixture of 2000 cc of 95% alcohol and 152 cc of  $\text{HCl}$ ). Filter thru an asbestos Gooch and wash with the acidulated alcohol and then with 80% alcohol. Dry the Gooch for 1 hour in an electric oven and then weigh. Dissolve the potassium platonic chloride with hot  $\text{H}_2\text{O}$ , wash with 80% alcohol, and again place in a drying oven for an hour. Cool in a desiccator, weigh, and calculate to  $\text{K}_2\text{O}$ . Calculate to  $\text{KCl}$  and deduct from the weight of combined Na and K chlorides to obtain  $\text{NaCl}$ . Calculate and report the result as percentage of  $\text{Na}_2\text{O}$ .

## 32

ARSENIC<sup>12</sup>

Weigh 5 g of air-dry soil and transfer to a 200 cc Kjeldahl flask. Add 20 cc of  $\text{H}_2\text{SO}_4$  (arsenic free) and thoroly mix the acid with the soil by rotating the flask. Add 5 cc of  $\text{HNO}_3$  (arsenic free) and 0.1 g of  $\text{KClO}_3$  to the  $\text{H}_2\text{SO}_4$  soil mixture in the flask. Heat the flask gently at first, then gradually increase the heat until the soln boils, and digest at this temp. until all the organic matter is oxidized and the acid soln is clear. (For soils high in organic matter content it may be necessary to add other portions of  $\text{HNO}_3$  to oxidize all the C and obtain a clear soln.) Cool the flask. Dilute the soln with 50 cc of distilled  $\text{H}_2\text{O}$  and concentrate by boiling until  $\text{SO}_3$  fumes are given off; repeat this operation twice to expel all traces of the oxides of N. Dilute the soln in the flask with distilled  $\text{H}_2\text{O}$ , transfer to a 100 cc volumetric flask, cool, and make to mark. Take an aliquot and determine arsenic by the modified Gutzeit method, XXIX, 1.

SELENIUM<sup>13</sup>

## 33

## I. For soils

Pulverize the air-dried sample with a wooden roller until all portions other than rock fragments are disintegrated. Separate the rock fragments by use of a 2 mm

sieve. Sub-sample the sieved material to secure a representative sample of 50 g. Transfer the weighed material to a distilling flask equipped with a short condenser and a thistle safety tube. The apparatus should have all glass connections (Fig. 4). Add 100 cc of hydrobromic acid (HBr) to which has been added 2 cc of Br. Warm gently for 15 min. and distil into a 100 cc Erlenmeyer flask containing 5 cc of  $H_2O$ . Have the outlet of the distilling tube submerged. If, on gentle warming, a drop of Br does not collect beneath the  $H_2O$  in the receiver, add 2 cc of Br to the distilling flask thru the thistle tube and repeat the gentle warming. Distil 60 cc of distillate into the receiver. To the distillate in the Erlenmeyer add 25 cc of  $H_2O$  and cool in ice  $H_2O$ . Pass a slow stream of  $SO_2$  into the distillate until the Br is removed. Add 0.25 g of hydroxylamine hydrochloride ( $NH_2OH \cdot HCl$ ). Warm the Erlenmeyer on a steam bath at  $80^\circ$  for 15 min. and allow to stand overnight. The selenium will appear at the bottom of the Erlenmeyer as a rose-pink precipitate. Modify the further treatment according to the quantity of the precipitate as directed below.

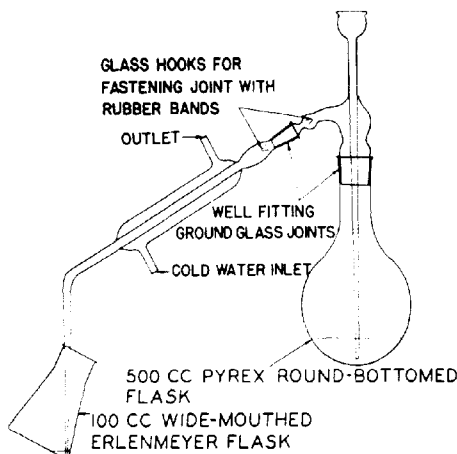


FIG. 4.—APPARATUS FOR DETERMINATION OF SELENIUM IN SOILS

(a) *Precipitate estimated as not greater than 0.5 mg.*—Filter thru a small asbestos Gooch crucible with suction. If a small quantity of oily material accompanies the precipitated selenium, wash the asbestos pad with 10 cc of 95% alcohol and then with 10 cc of  $H_2O$ . Redissolve the precipitated selenium from the asbestos pad with 10 cc of 48% HBr, which has been rendered a bright red by the addition of Br. Collect the filtrate by suction in a 25 cc volumetric flask and wash the pad with two portions of  $H_2O$ . Decolorize the filtered soln with  $SO_2$  and then add 1 cc of a soln containing 100 mg of  $NH_2OH \cdot HCl$  and 25 mg of gum arabic per cc. Make to volume with distilled  $H_2O$ . Transfer the flask and contents to a steam bath and warm at  $80^\circ$  for 30 min.; then cool to room temp., shake vigorously, and transfer to a 50 cc Nessler tube. Before the final precipitation of the selenium in the volumetric flask prepare a series of standards in 25 cc volumetric flasks by the addition of 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, and 0.7 mg of selenium as sodium selenate. Precipitate these standards after addition of HBr, Br,  $H_2O$ ,  $NH_2OH \cdot HCl$ , and gum arabic, and treat

precisely as the sample is treated. Compare the sample with the standard in any suitable color comparator and estimate the quantity of selenium present. Express the results obtained in p.p.m. of the air-dried soil.

(b) *When the quantity is below 1 p.p.m. (shown by preceding determination) and when greater delicacy is desired.*—Distil 100 g of the soil sample with HBr as directed under 33. When the distillation is complete, replace the soil sample in the distilling flask with a second 100 g sample. Add to the distillate, from the first distillation, 50 cc additional HBr and 2–4 cc of Br, together with 22 cc of  $\text{H}_2\text{SO}_4$ . Allow to stand and repeat the distillation as often as may be necessary to integrate the minute quantities of selenium until an adequate quantity for measurement is obtained.

(c) *When initial precipitation is in excess of 0.5 mg.*—Redissolve the washed precipitate in HBr colored with Br as directed under (a). Transfer the dissolved material to a 100 cc beaker and dilute with 20% HBr to a volume of 50 cc. Precipitate this soln with  $\text{SO}_2$  and add 0.25 g of  $\text{NH}_2\text{OH} \cdot \text{HCl}$ . Warm on a steam bath for 15 min. and allow to stand overnight at room temp. Filter on a weighed Gooch, dry at  $85^\circ$  for 4 hours, and weigh. The balance used must be sensitive to at least 0.05 mg.

34

#### II. For shales and sulfide ores

Place 200 cc of  $\text{HNO}_3$  in a 400 cc porcelain evaporating dish and heat to gentle boiling. Add slowly 10 g of the powdered sample. If the ensuing reaction is vigorous, add the sample in small quantities and allow the vigorous reaction to subside after each addition. When all the sample is added, add 50 cc of  $\text{H}_2\text{SO}_4$  and allow the mixture to stand on the water bath overnight. Transfer to a distilling flask and proceed as directed in 33, 33(a), or 33(b). If shales are free from sulfides, treat exactly as prescribed in 33.

35

#### QUALITATIVE TEST FOR SOIL REACTION

Place strips of neutral litmus paper in the bottom of a number of Petri dishes; over these lay 1 or 2 thicknesses of filter paper (free from acid); and place the soil, prepared as directed under 2(a), on the filter paper. With a horn spoon or clean spatula press the soil down firmly against the paper and add enough  $\text{H}_2\text{O}$  (tested and found neutral) to saturate the soil. Cover the dishes, allow to stand for 30 min., and then note the color of the test paper. A check Petri dish containing neutral litmus paper and filter paper, moistened with the same  $\text{H}_2\text{O}$ , is allowed to stand under the same conditions. The filter paper gives a uniform background and evenness of contact.

#### REACTION VALUES

36

#### DETERMINATION<sup>14</sup>

Use either the colorimetric or electrometric method, as found convenient. Determine the reaction values on fresh moist samples, using a soil-to-water ratio of 1:5, with intermittent agitation for 30 min.

37

#### METHOD OF STATEMENT

For simplicity and ease of interpretation use a dual system of statement, giving both pH values and their equivalents in arithmetically related numbers in parentheses, as tabulated (following page).

## METHODS OF ANALYSIS

SÖRENSEN OR pH VALUES	SPECIFIC ACIDITY OR HYDROGEN-ION CONCENTRATIONS	SÖRENSEN OR pH VALUES	SPECIFIC ALKALINITY OR HYDROXYL-ION CONCENTRATIONS
7.0	0.0	7.0	0.0
6.9	0.5	7.1	0.5
6.8	1.0	7.2	1.0
6.7	1.5	7.3	1.5
6.6	2	7.4	2
6.5	3	7.5	3
6.4	4	7.6	4
6.3	5	7.7	5
6.2	6	7.8	6
6.1	8	7.9	8
6.0	10	8.0	10
5.9	12.5	8.1	12.5
5.8	16	8.2	16
5.7	20	8.3	20
5.6	25	8.4	25
5.5	31.5	8.5	31.5
5.4	40	8.6	40
5.3	50	8.7	50
5.2	63	8.8	63
5.1	80	8.9	80
5.0	100	9.0	100
4.9	125	9.1	125
4.8	160	9.2	160
4.7	200	9.3	200
4.6	250	9.4	250
4.5	315	9.5	315

## 38

## NITRATE NITROGEN

Place 100 g of the air-dried soil, prepared as directed under 2(a), and 500 cc of  $H_2O$  in a suitable container, and agitate for 5 min. Add 1 g of  $CaO$  or 2 g of precipitated  $CaCO_3$ , agitate thoroly, allow to stand 10-20 min., and obtain a clear filtrate. If the filtrate contains 6 p.p.m. of  $Cl$ , or less, proceed as directed under **XXXVII, 17**, using 25 cc of the filtrate; if it contains more than 6 p.p.m.  $Cl$ , proceed as directed under **XXXVII, 19**, using 25 cc or a quantity that will contain not to exceed 0.1 mg of  $N$  in the form of nitrate. Report as percentage of nitrate  $N$ .

## 39

## ALKALI SALTS

To 100 g of soil in a 500 cc bottle, add 250 cc of  $H_2O$ . Stopper, shake thoroly, and allow to stand overnight. Filter thru a Pasteur-Chamberland filter. Evaporate 50 cc of the filtrate to dryness in a Pt dish on a steam bath, ignite at a low red heat to decompose organic matter, cool in a desiccator, and weigh for total salts. Dissolve the residue in the Pt dish in 10-15 cc of hot  $H_2O$ , transfer to a 50 cc volumetric flask, cool, and dilute to the mark.

For determination of  $Cl$ , titrate an aliquot of 10 cc against 0.1  $N$   $AgNO_3$  solu. Calculate and report the result as percentage of  $NaCl$ .

For determination of alkali carbonate, titrate an aliquot of 10 cc against 0.1  $N$   $HCl$ . Calculate and report the result as percentage of  $Na_2CO_3$ .

Determine sulfates by difference. If much  $CaSO_4$  is present, filter a 10 cc aliquot of the solu of the salts in hot  $H_2O$  thru a small filter, add 75 cc of alcohol, and digest for 3 hours; filter, wash with alcohol, and ignite; subtract the amount of  $CaSO_4$  from the amount of sulfates.

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## II. FERTILIZERS

### 1 DIRECTIONS FOR SAMPLING<sup>1</sup>—OFFICIAL

Each official sample sent to the laboratory shall consist of at least 1 lb. of the material taken in the following manner: Use a sampler that removes a core from the top to the bottom of the bag. Take cores from not less than 10% of the bags present unless this process necessitates cores from more than 20 bags, in which case take a core from 1 bag for each additional ton represented. If less than 100 bags, sample not less than 10 bags; if less than 10 bags, sample all bags. Thoroughly mix the portions taken on a clean oilcloth or paper, reduce by quartering to the quantity of sample required, and place in an air-tight container.

### 2 PREPARATION OF SAMPLE—OFFICIAL

Pass the entire sample submitted to the chemist thru a 10-mesh sieve before subdividing for analysis. Reduce the gross sample by quartering to a quantity sufficient for analytical purposes. Transfer the sample to a sieve having circular openings 1/25 in. (1 mm) in diameter and sift, breaking the lumps with a pestle. Grind the portion remaining on the sieve until all particles pass thru, grinding and sifting as rapidly as possible to avoid loss or gain of moisture during the operation. Mix thoroughly and preserve in tightly stoppered bottles.

### 3 MECHANICAL ANALYSIS OF BONE AND TANKAGE—OFFICIAL

Transfer 100 g of the original material to a sieve having circular openings 1/50 in. (0.5 mm) in diameter. Sift, breaking the lumps by means of a soft rubber pestle if the material has a tendency to cake. Weigh the coarse portion remaining on the sieve. Determine the fine portion by difference.

#### MOISTURE

### 4 *By Drying—Official*

Heat 2 g of the sample prepared as directed under 2 for 5 hours in a water oven at the temp. of boiling H<sub>2</sub>O (98–100°). In the case of potash salts, NaNO<sub>3</sub>, and (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, heat at about 130° to constant weight. Report the percentage loss in weight as moisture.

### *By Distillation with Toluene?—Tentative*

(Not applicable to urea, calcium nitrate, ammonium nitrate, and other salts containing water of crystallization, or that are volatile or decomposed at low temperature, and to mixtures containing such salts.)

### 5 APPARATUS

(a) *Distillation apparatus.*—250 cc Pyrex Erlenmeyer flask, receiving tube or trap graduated in 0.1 cc, and condenser. Ground-glass joints are preferable, but pressed cork may be used, XXVII, 3.

(b) *Tube brush attached to a long wire.*

### 6 DETERMINATION

Place in the flask sufficient sample to give 2–5 cc of H<sub>2</sub>O (weight should not exceed 20 g). Weigh rapidly to 1 mg. (Extremely hygroscopic materials should be

placed in covered weighing tubes, and the samples should be weighed out by difference.) If the sample is likely to bump, add enough dry sand to cover the bottom of the flask. Add 100 cc of toluene immediately and connect the flask to the trap and condenser. Pour 50 cc of toluene thru the condenser, filling the trap. Bring the mixture to a boil and distil slowly (about 2 drops per second), until most of the  $H_2O$  has passed over, then increase the rate of distillation to about 4 drops per second. When all the  $H_2O$  has apparently distilled, wash down the condenser by pouring 5–10 cc of toluene in at the top, and continue the distillation until no more  $H_2O$  will distil over. (The distillation for organic materials and salts that contain no water of crystallization may be completed within an hour, but such salts as calcium nitrate require a much longer time, 7–10 hours.) If  $H_2O$  remains in the condenser, remove it by brushing down with a tube brush attached to a long wire, washing down the condenser at the same time with toluene. Allow the receiving tube to come to room temp. Force any drops adhering to the sides of the tube into the  $H_2O$  column with a rubber band wrapped around a wire. Read the volume of  $H_2O$  and calculate the percentage of the sample, assuming the weight of 1 cc of  $H_2O$  to be 1 g at room temp.

It is necessary to have the condenser and receiving tube absolutely clean at the start to prevent adherence of  $H_2O$  to the glass. Clean with chromic-sulfuric acid, rinse with alcohol, and dry thoroly. Used toluene may be recovered by distillation from anhydrous  $CuSO_4$ .

#### TOTAL PHOSPHORIC ACID

##### *Gravimetric Method—Official*

7

#### REAGENTS

(a) *Molybdate soln.*—Dissolve 100 g of molybdic acid ( $MoO_3$ ) in a mixture of 144 cc of  $NH_4OH$  and 271 cc of  $H_2O$ . Pour this soln slowly and with constant stirring into a mixture of 489 cc of  $HNO_3$  and 1148 cc of  $H_2O$ . Keep the final mixture in a warm place for several days or until a portion heated to  $40^\circ$  deposits no yellow precipitate of  $NH_4$  phosphomolybdate. Decant the soln from any sediment and preserve in glass-stoppered vessels.

(b) *Ammonium nitrate soln.*—Dissolve 100 g of phosphate-free  $NH_4NO_3$  in  $H_2O$  and dilute to 1 liter.

(c) *Magnesia mixture.*—(1) Dissolve 11 g of  $MgO$  in  $HCl$  (1+4), avoiding an excess of the acid; add a little  $MgO$  in excess; boil a few minutes to precipitate  $Fe$ ,  $Al$ , and  $P_2O_5$ ; and filter. To the filtrate add 140 g of  $NH_4Cl$  and 130.5 cc of  $NH_4OH$  and dilute to 1 liter. Or, (2) dissolve 55 g of crystallized  $MgCl_2 \cdot 6H_2O$  in  $H_2O$ , add 140 g of  $NH_4Cl$  and 130.5 cc of  $NH_4OH$ , and dilute to 1 liter. Or, (3) dissolve 55 g of crystallized  $MgCl_2 \cdot 6H_2O$  in  $H_2O$ , add 140 g of  $NH_4Cl$ , and dilute to 870 cc. Add  $NH_4OH$  to each required portion of the soln just before using, at the rate of 15 cc per 100 cc of soln.

(d) *Ammonium hydroxide for washing* (1+9).—Should contain not less than 2.5% of  $NH_3$  by weight.

(e) *Magnesium nitrate soln.*—Dissolve 150 g of  $MgO$  in  $HNO_3$  (1+1), avoiding an excess of the acid; add a little  $MgO$  in excess, boil, filter from the excess of  $MgO$ ,  $Fe_2O_3$ , etc., and dilute to 1 liter.

8

#### PREPARATION OF SOLUTION

Treat 2 g of the sample by one of the methods given below. Cool the soln, dilute to 200 cc, mix, and pour on a dry filter.



(a) Dissolve in 30 cc of  $\text{HNO}_3$  and 3-5 cc of  $\text{HCl}$  and boil until the organic matter is destroyed. (Suitable for materials containing a small quantity of organic matter.)

(b) Dissolve in 15-30 cc of  $\text{HCl}$  and 3-10 cc of  $\text{HNO}_3$ . (Recommended for fertilizers containing much  $\text{Fe}$  or  $\text{Al}$  phosphate.)

(c) Evaporate with 5 cc of  $\text{MgNO}_3$ , ignite, and dissolve in  $\text{HCl}$ . (Suitable for organic material like cottonseed meal alone or in mixtures.)

(d) Boil with 20-30 cc of  $\text{H}_2\text{SO}_4$  in a 200 cc flask, adding 2-4 g of  $\text{NaNO}_3$  or  $\text{KNO}_3$  at the beginning of the digestion and a small quantity after the soln has become nearly colorless, or adding the nitrate in small portions from time to time. When the soln is colorless, add 150 cc of  $\text{H}_2\text{O}$  and boil for a few minutes. (Generally applicable to materials or mixtures containing large quantities of organic matter. With cottonseed meals and materials of like nature it is best to add first about 5 cc of  $\text{HNO}_3$  and then the  $\text{H}_2\text{SO}_4$ .) Before adding the nitrate, allow the mixture to digest, at a gentle heat if necessary, until the violence of the reaction is over.

## 9

## DETERMINATION

Pipet an aliquot of the prepared soln corresponding to 0.25 g, 0.50 g, or 1 g, into a 250 cc beaker; add  $\text{NH}_4\text{OH}$  in slight excess, and barely dissolve the precipitate formed with a few drops of  $\text{HNO}_3$ , stirring vigorously. If  $\text{HCl}$  or  $\text{H}_2\text{SO}_4$  has been used as a solvent, add about 15 g of crystalline  $\text{NH}_4\text{NO}_3$  or a soln containing that quantity. To the hot soln add 70 cc of the molybdate soln for every decigram of  $\text{P}_2\text{O}_5$  present. Digest at about  $65^\circ$  for 1 hour, and determine whether or not the  $\text{P}_2\text{O}_5$  has been completely precipitated by adding more molybdate soln to the clear supernatant liquid. Filter, and wash with cold  $\text{H}_2\text{O}$  or preferably with the  $\text{NH}_4\text{NO}_3$  soln. Dissolve the precipitate on the filter with  $\text{NH}_4\text{OH}$  (1+1) and hot  $\text{H}_2\text{O}$  and wash into a beaker to a volume of not more than 100 cc. Neutralize with  $\text{HCl}$ , using litmus paper or bromothymol blue as an indicator; cool; and from a buret add slowly (about 1 drop per second), stirring vigorously, 15 cc of the magnesia mixture for each decigram of  $\text{P}_2\text{O}_5$  present. After 15 min. add 12 cc of  $\text{NH}_4\text{OH}$ . Let stand until the supernatant liquid is clear (usually 2 hours), filter, wash the precipitate with  $\text{NH}_4\text{OH}$  (1+9) until the washings are practically free from chlorides, dry, burn at a low heat, and then ignite to constant weight, preferably in an electric furnace, at  $950-1000^\circ$ ; cool in a desiccator, and weigh as  $\text{Mg}_2\text{P}_2\text{O}_7$ . Calculate and report the result as percentage of  $\text{P}_2\text{O}_5$ .

*Volumetric Method—Official*

## 10

## REAGENTS

(a) *Molybdate soln.*—To 100 cc of molybdate soln, 7(a), add 5 cc of  $\text{HNO}_3$ . Filter this soln immediately before using.

(b) *Standard sodium or potassium hydroxide soln.*—Dilute 323.81 cc of  $N$  alkali, free from carbonates, to 1 liter; 100 cc of the soln should neutralize 32.38 cc of  $N$  acid; 1 cc = 1 mg or 1% of  $\text{P}_2\text{O}_5$  on a basis of 0.1 g of substance.

(c) *Standard acid soln.*—Prepare a soln of  $\text{HCl}$  or of  $\text{HNO}_3$ , corresponding to the strength of (b), or to  $\frac{1}{2}$  of this strength, and standardize by titration against that soln, using the phenolphthalein indicator.

(d) *Phenolphthalein indicator.*—Dissolve 1 g of phenolphthalein in 100 cc of alcohol, 95% by volume.

## 11

## PREPARATION OF SOLUTION

Treat 2 g of the sample as directed under 8(a), (b), (c) or (d), preferably (a) when these acids are a suitable solvent, and dilute to 200 cc with  $\text{H}_2\text{O}$ .

## 12

## DETERMINATION

(a) For percentages up to 5 use an aliquot corresponding to 0.4 g of substance; for percentages between 5 and 20 use an aliquot corresponding to 0.2 g of substance; and for percentages above 20 use an aliquot corresponding to 0.1 g of substance. Add 5–10 cc of  $\text{HNO}_3$ , depending on the method of soln (or the equivalent in  $\text{NH}_4\text{NO}_3$ ); add  $\text{NH}_4\text{OH}$  until the precipitate that forms dissolves but slowly on stirring vigorously, dilute to 75–100 cc, and adjust to a temp. of 25–30°. For percentages below 5, add 20–25 cc of the freshly filtered molybdate soln; for percentages between 5 and 20 add 30–35 cc of the molybdate soln; and for percentages greater than 20 add sufficient molybdate soln to insure complete precipitation. Place the soln in a shaking or stirring apparatus and shake or stir for 30 min. at room temp., decant *at once* thru a filter, and wash the precipitate twice by decantation with 25–30 cc portions of  $\text{H}_2\text{O}$ , agitating thoroly and allowing to settle. Transfer the precipitate to the filter and wash with cold  $\text{H}_2\text{O}$  until the filtrate from 2 fillings of the filter yields a pink color upon the addition of phenolphthalein and 1 drop of the standard alkali. Transfer the precipitate and filter to the beaker or precipitating vessel, dissolve the precipitate in a small excess of the standard alkali, add a few drops of phenolphthalein indicator, and titrate with the standard acid.

(b) *Not applicable in the presence of sulfates.*<sup>3</sup>—Proceed as directed under (a) to the point where the soln is diluted to 75–100 cc. Then heat in a water bath to 45–50°, add the molybdate soln at the rate of 75 cc for each decigram of  $\text{P}_2\text{O}_5$  present, and allow the mixture to remain in the bath, stirring occasionally for 30 min. Decant *at once* thru a filter, wash, and titrate as directed under (a).

## WATER-SOLUBLE PHOSPHORIC ACID

## 13

*Gravimetric Method—Official*

Place 1 g of the sample on a 9 cm filter and wash with successive small portions of  $\text{H}_2\text{O}$ , allowing each portion to pass thru before adding more, until the filtrate measures about 250 cc. If the filtrate is turbid, add 1–2 cc of  $\text{HNO}_3$ . Dilute to a convenient volume, mix well, and proceed as directed under 9.

## 14

*Volumetric Method—Official*

Treat the sample as directed under 13. To an aliquot of the soln corresponding to 0.1, 0.2 or 0.4 g, add 10 cc of  $\text{HNO}_3$ , nearly neutralize with  $\text{NH}_4\text{OH}$ , dilute to 60 cc, and proceed as directed under 12.

## CITRATE-INSOLUBLE PHOSPHORIC ACID (MODIFIED FRESSENIUS-GLADDING METHOD)—OFFICIAL

## 15

## REAGENT

*Ammonium citrate soln.*<sup>4</sup>—Should have a specific gravity of 1.09 at 20° and a pH of 7.0 as determined by the electrometric method with the hydrogen electrode or by the colorimetric method with phenol red. When using the colorimetric method proceed as follows:

Dissolve 370 g of crystallized citric acid in 1500 cc of  $\text{H}_2\text{O}$  and nearly neutralize by adding 345 cc of  $\text{NH}_4\text{OH}$  soln (28–29%  $\text{NH}_3$ ). If the concentration of the  $\text{NH}_4\text{OH}$  is less than 28%, add a correspondingly larger volume and dissolve the citric acid in a correspondingly smaller volume of  $\text{H}_2\text{O}$ . Cool, and make exactly neutral as follows:

Transfer 10 cc of the citrate soln to one of the standard test tubes of a hydrogen-

ion comparator set with color standards and add 0.5 cc of a 0.02% soln of phenol red or a sufficient volume to give the same concentration of indicator as used in the color standards. Add from a graduated pipet a few drops of  $\text{NH}_4\text{OH}$  (1+7), mix, compare the color by use of the comparator with that of the color standards of the same indicator, add more  $\text{NH}_4\text{OH}$ , if necessary, and repeat the test until the color matches that of the color standard corresponding to a pH of 7.0. If the  $\text{NH}_4\text{OH}$  added is in excess of that required to give a pH of 7.0, discard the soln and repeat the test, using a smaller quantity of  $\text{NH}_4\text{OH}$ . From the quantity of  $\text{NH}_4\text{OH}$  soln required to produce in the sample a color that exactly matches the standard, calculate the quantity of  $\text{NH}_4\text{OH}$  required to neutralize the rest of the soln. Add this calculated quantity of  $\text{NH}_4\text{OH}$  and check its reaction by repeating the test as before with the addition of a small quantity of  $\text{NH}_4\text{OH}$  or of a citric acid soln as may be required. When the color matches, dilute the soln, if necessary, to a density of 1.09 at 20°. (The volume will be about 2 liters.) Keep in tightly stoppered bottles and check pH from time to time.

Phenol red is recommended in place of bromothymol blue as the salt effect due to the presence of the  $\text{NH}_4$  citrate soln gives a pH reading with the latter indicator that is approximately 0.20 unit too high. When bromothymol blue is used, subtract 0.20 from the observed reading to obtain the true reading.

The other reagents and solns are described under 7 and 10.

## 16

### DETERMINATION<sup>5</sup>

(a) *Acidulated samples*.—Heat 100 cc of the  $\text{NH}_4$  citrate soln to 65° in a 250 cc flask placed in a water bath, keeping the flask loosely stoppered to prevent evaporation. Keep the level of the  $\text{H}_2\text{O}$  in the bath above that of the citrate soln in the flask. When the temp. of the citrate soln has reached 65°, drop into it the filter containing the residue from the water-soluble  $\text{P}_2\text{O}_5$ , 13, close the flask tightly with a smooth rubber stopper and shake vigorously until the filter paper is reduced to a pulp, relieving the pressure by momentarily removing the stopper. Return the flask to the bath and maintain its contents at exactly 65°. Shake the flask every 5 min. At the expiration of exactly 1 hour from the time the filter and the residue were introduced, remove the flask from the bath and immediately filter the contents as rapidly as possible thru a Whatman filter paper No. 5 or other paper of equal speed and retentiveness. It is recommended that filtration be made with suction with the use of a Büchner funnel or ordinary glass funnel with a Pt or other cone. Wash with  $\text{H}_2\text{O}$  at 65° until the volume of the filtrate is about 350 cc, allowing time for thoro draining before adding new portions of  $\text{H}_2\text{O}$ . If the sample gives a cloudy filtrate, wash with a 5% soln of  $\text{NH}_4\text{NO}_3$ . Determine the  $\text{P}_2\text{O}_5$  in the citrate-insoluble residue by one of the following methods: (1) Dry the filter and its contents, transfer to a crucible, ignite until all organic matter is destroyed, and digest with 10–15 cc of  $\text{HCl}$  until all phosphate is dissolved; (2) transfer the wet filter with contents to a 200 cc flask, add 30–35 cc of  $\text{HNO}_3$  and 5–10 cc of  $\text{HCl}$ , and boil until all phosphate is dissolved; or (3) treat the filter and its contents as directed under 8(c) or (d). Dilute the soln to 200 cc, mix well, filter thru a dry filter, and proceed as directed under 9 or 12.

(b) *Non-acidulated samples other than basic slag*.—Place 1 g of the sample on a 9 cm filter paper. Without previous washing with  $\text{H}_2\text{O}$ , proceed as directed under (a) and determine  $\text{P}_2\text{O}_5$  as directed under 9 or 12. If the substance contains much animal matter (bone, fish, etc.), dissolve the residue insoluble in  $\text{NH}_4$  citrate by one of the processes described under 8(c) or (d).

## 17 CITRATE-SOLUBLE PHOSPHORIC ACID—OFFICIAL

Subtract the sum of the water-soluble and citrate-insoluble  $P_2O_5$  from the total to obtain the citrate-soluble  $P_2O_5$ .

## 18 DETECTION OF NITRATES—OFFICIAL

Mix 5 g of the fertilizer with 25 cc of hot  $H_2O$ , and filter. To 1 volume of this soln add 2 volumes of  $H_2SO_4$ , free from  $HNO_3$  and oxides of N, and allow the mixture to cool. Add a few drops of a concentrated soln of ferrous sulfate in such a manner that the fluids will not mix. If nitrates are present, the junction shows at first a purple, afterwards a brown, color or if only a very minute quantity is present, a reddish color. To another portion of the soln add 1 cc of a 1% soln of  $NaNO_3$  and test as before to determine whether sufficient  $H_2SO_4$  was added in the first test.

## ORGANIC AND AMMONIACAL NITROGEN ONLY

*Kjeldahl Method—Official*

## 19

## REAGENTS

For ordinary work 0.5 N acid is recommended, but in determining very small quantities of N 0.1 N acid is recommended. In titrating mineral acids against a soln of  $NH_4OH$  use cochineal or methyl red as indicator.

(a) *Standard hydrochloric acid.*—Determine the absolute strength as follows:

PRELIMINARY TEST: Place a measured portion of the acid to be standardized in an Erlenmeyer flask and add an excess of  $CaCO_3$  to neutralize free acid and a few drops of a 10% soln of  $K_2CrO_4$  as indicator. Titrate with 0.1 N  $AgNO_3$  soln and note the exact quantity required to precipitate the chlorides.

FINAL DETERMINATION: To a measured portion of the acid to be standardized add from a buret 1 drop in excess of the required quantity of  $AgNO_3$  soln as determined by the preliminary test. Heat the mixture to boiling, protect from the light, and allow to stand until the precipitate is granular. Filter on a Gooch crucible, previously heated to  $140-150^\circ$  and weighed; wash with hot  $H_2O$ , testing the filtrate to verify an excess of  $AgNO_3$ . Dry the  $AgCl$  at  $140-150^\circ$ , cool, and weigh.

(b) *Standard sulfuric acid.*—Determine the strength of the acid by precipitation with  $BaCl_2$  soln as follows: Dilute a measured quantity of the acid to be standardized to approximately 100 cc; heat to boiling; and add, dropwise, a 10% soln of  $BaCl_2$  until no further precipitation occurs. Continue the boiling for about 5 min., allow to stand for 5 hours or longer in a warm place, pour the supernatant liquid on a weighed Gooch crucible or an ashless filter, treat the precipitate with 25–30 cc of boiling  $H_2O$ , transfer to the filter, and wash with boiling  $H_2O$  until the filtrate is free from chlorides. Dry, ignite over a Bunsen burner, and weigh as  $BaSO_4$ .

(c) *Standard alkali soln.*—A 0.1 N soln is recommended. Accurately determine the strength of this soln by titration against the standard acid prepared as directed under (a) or (b), or proceed as directed under 4,<sup>6</sup> Appendix 1.

(d) *Sulfuric acid.*—Should contain 93–96%  $H_2SO_4$  and be free from nitrates and  $(NH_4)_2SO_4$ .

(e) *Metallic mercury, or mercuric oxide.*—The mercuric oxide should be prepared in the wet way, but not from  $Hg(NO_3)_2$ .

(f) *Sulfide, or thiosulfate soln.*—Dissolve 40 g of commercial  $K_2S$  in 1 liter of  $H_2O$ . A soln of 40 g of  $Na_2S$  or 80 g of  $Na_2S_2O_3 \cdot 5H_2O$  in a liter may be used.

(g) *Sodium hydroxide soln.*—Dissolve approximately 450 g of commercial  $NaOH$ , free from nitrates, in 1 liter of  $H_2O$ . This soln should have a sp. gr. of 1.43–1.48.

(h) *Cochineal indicator.*—Digest 3 g of pulverized cochineal in a mixture of 50 cc

of 95% alcohol and 200 cc of  $H_2O$  for 1 or 2 days at ordinary temp., agitating frequently, and then filter.

(i) *Methyl red indicator*.—Dissolve 1 g of methyl red (dimethylaminoazobenzene-orthocarboxylic acid) in 50 cc of 95% alcohol, dilute to 100 cc with  $H_2O$ , and filter if necessary.

Test reagents before using by a blank determination with sugar, which insures partial reduction of any nitrates present.

## 20

## APPARATUS

(a) *Kjeldahl flasks for both digestion and distillation*.—Total capacity of about 550 cc, made of hard, moderately thick, and well-annealed glass.

(b) *Distillation flask*.—Use any suitable flask of about 550 cc capacity, fitted with a rubber stopper thru which passes the lower end of a Kjeldahl connecting bulb to prevent NaOH being carried over mechanically during the distillation. Use a bulb 5 or 6 cm in diameter, and connect the upper end of the bulb tube to the condenser tube by means of rubber tubing.

## 21

## DETERMINATION

Place 0.7–3.5 g, according to the N content of the material to be analyzed, in a digestion flask. Add approximately 0.7 g of  $HgO$ , or its equivalent in metallic Hg, and 20–30 cc of  $H_2SO_4$  (0.1–0.3 g of crystallized  $CuSO_4$  may also be used in addition to the Hg, or in many cases, in place of it). Place the flask in an inclined position and heat below the boiling point of the acid until frothing has ceased. (A small piece of paraffin may be added to prevent extreme foaming.) Increase the heat until the acid boils briskly and digest for a time after the mixture is colorless or nearly so, or until oxidation is complete (approximately 2 hours).

After cooling, dilute with about 200 cc of  $H_2O$ , and add a few pieces of granulated Zn or pumice stone to prevent bumping, and 25 cc of the  $K_2S$  or  $Na_2S$  or  $Na_2S_2O_3$  soln with shaking. (If  $Na_2S_2O_3$  is used, it should first be mixed with the NaOH so that they may be added together. When no Hg or  $HgO$  is used the addition of  $K_2S$  or  $Na_2S$  or  $Na_2S_2O_3$  soln is unnecessary.) Add sufficient NaOH soln to make the reaction strongly alkaline (50 cc is usually sufficient), pouring it down the side of the flask so that it does not mix at once with the acid soln. Connect the flask to the condenser by means of a Kjeldahl connecting bulb, taking care that the tip of the condenser extends below the surface of the standard acid in the receiver; mix the contents by shaking and distil until all  $NH_3$  has passed over into a measured quantity of the standard acid. (The first 150 cc of the distillate generally contains all the  $NH_3$ .) Titrate with standard alkali soln, using the methyl red or cochineal indicator.

*Gunning Method—Official*

## 22

## REAGENTS AND APPARATUS

Use the reagents and apparatus described under 19 and 20.

## 23

## DETERMINATION

Place 0.7–3.5 g, according to the N content of the material to be analyzed, in a digestion flask. Add 10 g of powdered  $K_2SO_4$  or anhydrous  $Na_2SO_4$  and 15–25 cc (ordinarily about 20 cc) of  $H_2SO_4$  (0.1–0.3 g of crystallized  $CuSO_4$  may also be added). Conduct the digestion as in the Kjeldahl process, starting with a temp. below the boiling point and increasing the heat gradually until frothing ceases.

Digest for a time after the mixture is colorless or nearly so, or until oxidation is complete (usually 2 hours). Complete as directed under 21, but do not add  $K_2S$  or  $Na_2S$  or  $Na_2S_2O_3$ . In making the mixture alkaline before distilling add litmus paper or a few drops of phenolphthalein indicator. (The pink color given by phenolphthalein, indicating an alkaline reaction, is destroyed by a considerable excess of strong fixed alkali.)

*Kjeldahl-Gunning-Arnold Method—Official*

24

## REAGENTS AND APPARATUS

Use the apparatus, reagents, and solns described under 19 and 20.

25

## DETERMINATION

Place 0.7-3.5 g, according to the N content of the material to be analyzed, in a digestion flask. Add 15-18 g of  $K_2SO_4$  or anhydrous  $Na_2SO_4$ , 1 g of  $CuSO_4$  or approximately 0.7 g of  $HgO$  (or its equivalent in metallic Hg), and 25 cc of the  $H_2SO_4$ . Heat the mixture gently until frothing ceases, then boil briskly, and continue the digestion for a time after the mixture is colorless or nearly so, or until oxidation is complete (approximately 2 hours). Cool, add about 200 cc of  $H_2O$ , and if  $HgO$  or metallic Hg has been used, add also 50 cc of the  $K_2S$  or  $Na_2S$  or  $Na_2S_2O_3$  soln. Make strongly alkaline with the  $NaOH$  and complete the determination as directed under 21.

## TOTAL NITROGEN

*Kjeldahl Method Modified to Include the Nitrogen of Nitrates—Official*

26

## REAGENTS AND APPARATUS

Use the apparatus, reagents, and solns described under 19 and 20.

27

## DETERMINATION

Place 0.7-3.5 g, according to the N content of the material to be analyzed, in a Kjeldahl digestion flask. (1) Add 30 cc of the  $H_2SO_4$  containing 1 g of commercial salicylic acid, shake until thoroly mixed, allow to stand for at least 30 min. with frequent shaking or until complete soln results, and then add 5 g of crystallized  $Na_2S_2O_3$  and digest as directed below; or, (2) add to the substance 30 cc of  $H_2SO_4$  containing 2 g of the salicylic acid, allow to stand at least 30 min. with frequent shaking or until complete soln results, and then add gradually 2 g of Zn dust (an impalpable powder—granulated Zn or filings not satisfactory), shaking the contents of the flask at the same time, and digest as follows:

Heat over a low flame until all danger from frothing has passed. Then increase the heat until the acid boils briskly and continue the boiling until white fumes no longer escape from the flask (5-10 min.). Add approximately 0.7 g of  $HgO$ , or its equivalent in Hg, and continue the boiling until the liquid in the flask is colorless, or nearly so. If the contents of the flask are likely to become solid before this point is reached, add 10 cc more of  $H_2SO_4$ . Complete the determination as directed under 21. Test the reagents by blank determinations.

*Gunning Method Modified to Include the Nitrogen of Nitrates—Official*

28

## REAGENTS AND APPARATUS

Use the apparatus, reagents, and solns described under 19 and 20.

## 29

## DETERMINATION

Place 0.7–3.5 g, according to the N content of the material to be analyzed, in a digestion flask. Add 30 cc of  $\text{H}_2\text{SO}_4$  containing 1 g of commercial salicylic acid. Shake until thoroly mixed and allow to stand, shaking frequently, for at least 30 min., or until complete soln results. Add 5 g of  $\text{Na}_2\text{S}_2\text{O}_3$  and heat the soln for 5 min.; cool, add 10 g of  $\text{K}_2\text{SO}_4$  or anhydrous  $\text{Na}_2\text{SO}_4$ , heat very gently until foaming ceases, and proceed as directed under 23.

## AMMONIACAL NITROGEN

## 30

*Magnesium Oxide Method—Official*

Place 0.7–3.5 g, according to the  $\text{NH}_3$  content of the material to be analyzed, in a distillation flask with about 200 cc of  $\text{H}_2\text{O}$  and 2 g or more of  $\text{MgO}$  free from carbonates. Connect the flask with a condenser by means of a Kjeldahl connecting bulb, distil 100 cc of the liquid into a measured quantity of standard acid, and titrate with standard alkali, using cochineal or methyl red indicator, 19(h) and (i).

## NITRATE AND AMMONIACAL NITROGEN

## 31

*Reduced Iron Method—Official*

(Applicable in the presence of organic matter, but not in the presence of calcium cyanamide and urea.)

Place 0.7 or 1 g of the sample in a 500 cc flask, add about 30 cc of  $\text{H}_2\text{O}$  and 2–3 g of reduced Fe, and allow the mixture to stand sufficiently long to insure soln of the soluble nitrates and  $\text{NH}_4$  salts. Add 10 cc of  $\text{H}_2\text{SO}_4$  (1+1) and shake thoroly. Place a long-stemmed funnel in the neck of the flask to prevent mechanical loss, and allow to stand until the violence of the reaction has moderated. Heat the soln slowly, boil for 5 min., and cool. Add about 100 cc of  $\text{H}_2\text{O}$ , a little paraffin, and 7–10 g of  $\text{MgO}$ , free or nearly free from carbonates. By means of a Kjeldahl connecting bulb connect the flask with a condenser, such as is used in the Kjeldahl method, and boil the mixture nearly to dryness (about 40 min.); collect the  $\text{NH}_3$  in a measured quantity of standard acid and titrate with standard alkali soln, using cochineal or methyl red indicator, 19(h) and (i). The N obtained represents the nitrates plus the  $\text{NH}_4$  salts contained in the sample.

In the analysis of nitrate salts proceed as above, but use 25 cc of the nitrate soln, equivalent to 0.25 g of the sample, with 5 g of reduced Fe. After boiling, add 75 cc of  $\text{H}_2\text{O}$  and an excess of  $\text{NaOH}$  soln and complete the determination as directed above.

## 32

*Ferrous Sulfate-Zinc-Soda Method—Official*

(Not applicable in the presence of organic matter, calcium cyanamide and urea.)

Place 0.35, 0.5, or 0.7 g of the sample in a 600–700 cc flask and add 200 cc of  $\text{H}_2\text{O}$ , 5 g of powdered Zn, 1–2 g of ferrous sulfate, and 50 cc of  $\text{NaOH}$  soln (sp. gr. 1.33). Connect with the distilling apparatus, distil, collect the distillate in the usual way in 0.1 N  $\text{H}_2\text{SO}_4$ , and titrate with standard alkali, using cochineal or methyl red indicator, 19(h) and (i).

## 33

*Devarda Method—Official*

(Not applicable in the presence of organic matter, calcium cyanamide and urea.)

Place 0.5 g of the sample in a 600–700 cc flask and add 300 cc of  $\text{H}_2\text{O}$ , 3 g of

Devarda alloy and 5 cc of NaOH soln (42% by weight), pouring the latter down the side of the flask so that it does not mix at once with the contents. Connect, by means of a Davisson<sup>8</sup> or other suitable scrubbing bulb that will prevent the passing over of any portion of the spray, with a condenser, the tip of which always extends beneath the surface of the standard acid in the receiving flask. Mix the contents of the distilling flask by rotating. Heat slowly at first and then at such a rate that the 250 cc of distillate required will pass over in 1 hour. Collect the distillate in a measured quantity of standard acid, 19(a) or (b), and titrate with standard alkali soln, 19(c), using cochineal or methyl red indicator, 19(h) or (i).

## NITRATE NITROGEN

34

*Robertson Method<sup>9</sup>—Official*

(Applicable in the presence of calcium cyanamide and urea.)

- (a) Determine the total N as directed under 27 or 29.
- (b) Weigh out 2.0 g of the fertilizer mixture on a filter, wash with H<sub>2</sub>O to nearly 200 cc in a graduated flask, and make up to volume. If the fertilizer mixture is greasy or does not wet easily, moisten the sample with 7 cc of ethyl alcohol and continue the washing to nearly 200 cc. Determine the N in the residue as directed under 21, 23, or 25.
- (c) Determine the ammoniacal N in 50 cc of the filtrate as directed under 30.
- (d) Place another 50 cc portion of the filtrate in a 500 cc Kjeldahl flask and add 2 g of ferrous sulfate and 20 cc of H<sub>2</sub>SO<sub>4</sub>. (If the total N is over 5%, use 5 g of ferrous sulfate.) Digest over a hot flame until all the H<sub>2</sub>O is evaporated and white fumes appear and continue the digestion for at least 10 min. to drive off the nitrate N. If severe bumping occurs, add 10–15 glass beads. Add 0.65 g of Hg or its equivalent of HgO and digest until all the organic matter is oxidized. Cool, dilute, add the K<sub>2</sub>S soln, and complete the determination as directed under 25. (A pinch of a mixture of Zn dust and granular Zn (20-mesh) should be added to each flask before distillation to prevent bumping.)

Total N(a) – water-insoluble N(b) = water-soluble N. Water-soluble N – the N obtained in (d) = the nitrate N.

Ammoniacal N + nitrate N = mineral N. Total N – mineral N = organic N.

35

*Jones Modification of the Robertson Method*

(Applicable when a determination of water-soluble nitrogen is not needed.)

Weigh 0.5 g of the sample into a Kjeldahl flask. Add 50 cc of H<sub>2</sub>O and rotate gently; then add 2 g of ferrous sulfate and rotate. Add 20 cc of H<sub>2</sub>SO<sub>4</sub>. Digest over a hot flame. When the H<sub>2</sub>O is evaporated and white fumes appear, add 0.65 g of Hg and proceed as directed under 21. Cool, dilute, and distil as usual. The total N – the N thus found = the nitrate N.

36 WATER-INSOLUBLE NITROGEN IN CYANAMIDE<sup>10</sup>—OFFICIAL, FIRST ACTION

Weigh 2 g of the finely ground cyanamide and place in a mortar. Gradually add about 70 cc of distilled H<sub>2</sub>O while stirring with the pestle and grind thoroly. Transfer the mixture to a beaker, washing out the mortar with distilled H<sub>2</sub>O. Filter on a 11 cm filter paper. When all the cyanamide has been transferred to the paper, wash with an additional 250 cc of distilled H<sub>2</sub>O, allowing time for complete drainage before adding more H<sub>2</sub>O. Remove the filter paper and residue to a digestion flask. Determine the insoluble nitrogen in the residue as directed under 21, 23, or 25.



## NITROGEN ACTIVITY

37 *Determination of Water-Insoluble Organic Nitrogen—Official*

Place 1 or 1.4 g of the material on an 11 cm filter paper wet with alcohol and wash with  $H_2O$  at room temp. until the filtrate measures 250 cc. If the material is oily or does not wet readily with  $H_2O$ , wash with 5 cc of alcohol and then with the requisite quantity of  $H_2O$ . Dry, and determine N in the residue as directed under 21 or 23, making a correction for water-insoluble N of the filter paper, if necessary.

38 *Removal of Water-Soluble Nitrogen—Official*

(a) *Mixed Fertilizers*.—Place the quantity of fertilizer equivalent to 50 mg of water-insoluble organic N, 37, on an 11 cm filter paper wet with alcohol and wash with  $H_2O$  at room temp. until the filtrate measures 250 cc. If the material is oily or does not wet readily with  $H_2O$ , wash with 5 cc of alcohol and then with the requisite quantity of  $H_2O$ . When it is found necessary to use 4 g or more of the material, weigh the required quantity into a small beaker, wet with alcohol, wash by decantation, finally transfer to the filter, and finish the extraction as directed previously.

(b) *Raw Materials*.—Place the quantity of material equivalent to 50 mg of water-insoluble N, 37, in a small mortar; add about 2 g of powdered rock phosphate, mix thoroly, transfer to a filter paper wet with alcohol, and wash with  $H_2O$  at room temp. until the filtrate measures 250 cc. If the material is oily or does not wet readily with  $H_2O$ , wash with 5 cc of alcohol and then with the requisite quantity of  $H_2O$ .

39 *Water-Insoluble Organic Nitrogen Soluble in Neutral Permanganate—Official*

Using 25 cc of tepid  $H_2O$ , transfer the insoluble residue obtained in 38 to a 400 cc Griffin low-form beaker; add 1 g of  $Na_2CO_3$ , mix, and add 100 cc of a 2% soln of  $KMnO_4$ . Cover with a watch-glass and immerse for 30 min. in a steam or hot water bath, keeping the liquid in the beaker below that of the  $H_2O$  in the bath. Stir twice at intervals of 10 min. At the end of 30 min. remove from the bath, add immediately 100 cc of cold  $H_2O$ , and filter thru a heavy 15 cm folded filter. Wash with small quantities of cold  $H_2O$  until the filtrate measures about 400 cc. Determine N in the residue and the filter, as directed under 21 or 23, correcting for the N contained in the filter. The N thus obtained is the inactive water-insoluble organic N. The N obtained under 37—the percentage of N found = the water-insoluble organic N soluble in neutral permanganate.

*Water-Insoluble Organic Nitrogen Distilled from Alkaline Permanganate<sup>1</sup>—Official*

## 40

## REAGENTS

(a) *Stock soln of potassium permanganate*.—Dissolve 50 g of  $KMnO_4$  in a liter of  $H_2O$ . Dissolve 0.5 g of Na oxalate in 300 cc of  $H_2O$  and 10 cc of  $H_2SO_4$ . Heat to 75–80° and titrate with the  $KMnO_4$  soln, using a Mohr pipet or an all-glass buret to contain the permanganate soln.  $235.89 \div$  by the result of the titration in cc = the concentration of  $KMnO_4$  in g per liter. Adjust the concentration to 50 g per liter, protect from the light, and store at a temp. above 15°.

(b) *Stock soln of sodium hydroxide*.—Dissolve 300 g of NaOH in 1 liter of  $H_2O$ . Cool before using.

(c) *Alkaline permanganate soln*.—Mix equal quantities of the stock solns (a) and

(b) and add 10 cc of  $H_2O$  for each liter of soln that the mixture is calculated to make. Use this soln immediately, as it is unstable.

## 41

## DETERMINATION

Dry the residue remaining after treatment of the material as directed in 38 at a temp. not exceeding  $80^\circ$  and transfer from the filter to a 500–600 cc Kjeldahl distillation flask, loosening adhering particles by rubbing gently with a stiff brush but avoiding the transfer of portions of the brush or of paper fibers. Add 20 cc of  $H_2O$ , 15–20 small glass beads or fragments of pumice stone, a drop of mineral lubricating oil weighing not more than 50 mg, and 100 cc of the alkaline permanganate soln. Connect with an upright condenser to the lower end of which has been attached a 100 cc graduated cylinder containing standard acid and so arranged as to receive the distillate below the surface of the acid or otherwise trapped so as to prevent loss of  $NH_3$  fumes. Digest slowly with a very low flame for 30 min., barely below distillation point, using coarse wire gauze and asbestos paper between the flask and flame. Gradually raise the temp., and after all danger from frothing has passed distil 95 cc in 60 min. (plus or minus 5 min.), controlling the distillation to obtain approximately 24 cc of distillate in each 15 min. period. Conduct the first part of the distillation over a bare flame but use wire gauze 10 min. before completion to avoid breaking the flask. Transfer the distillate to an Erlenmeyer flask or to a beaker and titrate with standard alkali, using cochineal or methyl red indicator. When a tendency to froth is noticed, lengthen the digestion period, and no trouble will be experienced when the distillation is begun. During the digestion gently rotate the flask occasionally, particularly if the material shows a tendency to adhere to the sides.

The N thus obtained is the active water-insoluble organic N. If active water-insoluble N is found to be less than 55% of the total water-insoluble organic N present, it is recommended that a second portion of the sample be prepared as directed under 38. Dry the residue below  $80^\circ$ , transfer from the filter to a Kjeldahl flask as directed above, and determine the N as directed under 21 or 23. Recalculate the percentage of active water-insoluble N on the basis of the quantity of water-insoluble N thus found.

Previous to digestion with alkaline permanganate, the washed sample may be transferred from the filter to the flask by spreading the filter on a metal disk bent to form a trough that fits the palm of the hand, brushing the larger portion of the material into the flask with a spatula, and washing in the remainder with 20 cc of  $H_2O$  from a 20 cc pipet or small wash bottle. Do not add more  $H_2O$  before the digestion with alkaline permanganate, but, with this exception, proceed as with the transfer of the dried material.

## POTASH

*Method I. Lindo-Gladding<sup>12</sup>—Official*

## 42

## REAGENTS

(a) *Ammonium chloride soln.*—Dissolve 100 g of  $NH_4Cl$  in 500 cc of  $H_2O$ , add 5–10 g of pulverized  $K_2PtCl_6$ , and shake at intervals for 6–8 hours. Allow the mixture to settle overnight and filter. (The residue may be used for the preparation of a fresh supply.)

(b) *Platinum soln.*—A Pt soln containing the equivalent of 1 g of metallic Pt (2.1 g of  $H_2PtCl_6$ ) in every 10 cc. For materials containing less than 15% of  $K_2O$ , a Pt soln containing 0.2 g of metallic Pt (0.42 g of  $H_2PtCl_6$ ) in each 10 cc is recommended.

## 43

## PREPARATION OF SOLUTION

(a) *Mixed fertilizers*.—Place 2.5 g of the sample in a 250 cc volumetric flask, and add 125 cc of  $H_2O$  and 50 cc of saturated  $NH_4$  oxalate soln. Boil for 30 min., add a slight excess of  $NH_4OH$ , and after cooling dilute to 250 cc, mix, and pass thru a dry filter.

(b) *Potash salts: muriate and sulfate of potash, sulfate of potash and magnesia; and kainit*.—Dissolve 2.5 g and dilute to 250 cc without the addition of  $NH_4OH$  and  $NH_4$  oxalate. When substances that interfere, such as ammonia, lime, aluminum, etc., are present, proceed as directed in (a).

(c) *Organic compounds*.—To determine the total amount of  $K_2O$  in organic substances, such as cottonseed meal, tobacco stems, etc., saturate 10 g of the sample with  $H_2SO_4$  and ignite in a muffle at a low red heat to destroy organic matter. Add a little  $HCl$ , warm slightly in order to loosen the mass from the dish, transfer to a 500 cc volumetric flask, add  $NH_4OH$  and saturated  $NH_4$  oxalate soln, cool, dilute to 500 cc, mix, pass thru a dry filter, and proceed as directed under 44(a).

(d) *Ashes from wood, cotton hulls, etc.*—Boil 10 g of the sample with 300 cc of  $H_2O$  for 30 min., and add to the hot soln a slight excess of  $NH_4OH$  and then sufficient saturated  $NH_4$  oxalate soln to precipitate all the lime present. Cool, dilute to 500 cc, mix, and pass thru a dry filter.

## 44

## DETERMINATION

(a) *Mixed fertilizers*.—Evaporate nearly to dryness a 25 or 50 cc aliquot of soln 43(a) to which has been added sufficient potash-free normal  $NaOH$  (1-2 cc) to prevent the formation of free phosphoric acid during ignition; add 1 cc of  $H_2SO_4$  (1+1) and 6-8 granules of granulated sugar, evaporate to dryness, and ignite to whiteness at low temp. Maintain a dull red heat until the residue is perfectly white. Dissolve the residue in hot  $H_2O$ , using at least 20 cc for each decigram of  $K_2O$  present, and add a few drops of  $HCl$  and then an excess of the  $Pt$  soln. Evaporate on a water bath to a thick paste, avoiding exposure to  $NH_3$ . Treat the residue with approximately 6 cc of 80% alcohol, adding 0.6 cc of  $HCl$ . Filter on a Gooch crucible and wash the precipitate thoroly with 80% alcohol, both by decantation and on the filter, continuing the washing after the filtrate is colorless. Then wash 5 or 6 times with 10 cc portions of the  $NH_4Cl$  soln to remove impurities from the precipitate. Wash again thoroly with 80% alcohol and dry the precipitate for 30 min. at  $100^\circ$ . Weigh and calculate to  $K_2O$ . (The precipitate should be completely soluble in water.)

(b) *Muriate of potash*.—Acidify 50 cc of the soln prepared according to 43(b) with a few drops of  $HCl$ , add 10 cc of the  $Pt$  soln, and evaporate to a thick paste. Treat the residue as directed under (a). If  $NH_4OH$  and  $NH_4$  oxalate are used in the preparation of this soln, proceed as directed in (a).

(c) *Sulfate of potash, sulfate of potash and magnesia, and kainit*.—Acidify 50 cc of the soln prepared according to 43(b) with a few drops of  $HCl$  and add 15 cc of the  $Pt$  soln. Evaporate the mixture and proceed as directed under (a), but use 25 cc portions of the  $NH_4Cl$  soln. If  $NH_4OH$  and  $NH_4$  oxalate are used in the preparation of this soln, proceed as directed in (a) but use 25 cc portions of  $NH_4Cl$  soln.

(d) *Ashes from wood, cotton hulls, etc.*—Prepare the soln according to 43(d) and proceed as directed under (a), paying special attention to the last sentence.

For the conversion of  $K_2PtCl_6$  to  $KCl$  use the factor 0.3061; to  $K_2SO_4$ , 0.35843; to  $K_2O$ , 0.19376.

*Method II—Official*

(In the presence of soluble sulfates use preferably Method I.)

45

## REAGENTS

Use the reagents described under 42.

46

## PREPARATION OF SOLUTION

Prepare the soln as directed under 43(b), omitting in all cases the addition of  $\text{NH}_4\text{OH}$  and  $\text{NH}_4$  oxalate.

47

## DETERMINATION

Dilute 25 cc of the prepared soln (50 cc if less than 10% of  $\text{K}_2\text{O}$  is present) to 150 cc; heat to  $100^\circ$ ; and add, drop by drop, with constant stirring, a slight excess of a 10%  $\text{BaCl}_2$  soln. Without filtering, add in the same manner an excess of a saturated soln of  $\text{Ba}(\text{OH})_2$ . Filter while hot and wash until the precipitate is free from chlorides. Add to the filtrate 1 cc of  $\text{NH}_4\text{OH}$ , and then a saturated soln of  $(\text{NH}_4)_2\text{CO}_3$  until the excess of Ba is precipitated. Heat, and add, in fine powder, 0.5 g of pure oxalic acid or 0.75 g of  $\text{NH}_4$  oxalate. Filter, wash free from chlorides, evaporate the filtrate to dryness in a Pt dish, and ignite carefully over the free flame below a red heat until all volatile matter is driven off. Digest the residue with hot  $\text{H}_2\text{O}$ , filter thru a small filter, and dilute the filtrate, if necessary, to provide for each decigram of  $\text{K}_2\text{O}$  at least 20 cc of liquid. Acidify with a few drops of  $\text{HCl}$  and add an excess of the Pt soln. Evaporate on a water bath to a thick paste; treat the residue repeatedly with 80% alcohol, decanting thru a weighed Gooch crucible or other form of filter, transfer the precipitate to the filter, and wash thoroly with the 80% alcohol. Dry for 30 min. at  $100^\circ$  and weigh. If there is an appearance of foreign matter in the double salt, wash as directed under 44(a) with several portions of 10 cc each of the  $\text{NH}_4\text{Cl}$  soln.

WATER-SOLUBLE BORIC ACID<sup>13</sup>—OFFICIAL

48

## REAGENTS

(a) *Standard sodium hydroxide soln.*—Prepare this soln free from carbonates by first making a saturated soln (100 g of  $\text{NaOH}$  in 100 cc of  $\text{H}_2\text{O}$ ) in order to precipitate any  $\text{Na}_2\text{CO}_3$  present when the soln is allowed to stand in a vessel from which the  $\text{CO}_2$  of the air is excluded. Filter thru a hard filter that has been soaked in alcohol, dilute a portion with boiled and cooled  $\text{H}_2\text{O}$  to about 0.1 *N*, and accurately determine the strength of the soln by titration, as directed under 49(a), against 0.1 *N* boric acid soln.

(b) *Methyl red indicator.*—Dissolve 0.1 g of methyl red in 50 cc of 95% alcohol, dilute to 1 liter, and filter, if necessary.

49

## DETERMINATION

(a) *Mineral salts.*—Dissolve 5–10 g of the sample in 50–75 cc of hot  $\text{H}_2\text{O}$ , decomposing carbonates, if present, with a slight excess of  $\text{HCl}$ ; heat to boiling and add sufficient 10%  $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$  soln to precipitate the sulfates, using about 10 cc in excess. Add in small quantities sufficient powdered  $\text{Ba}(\text{OH})_2$  to make the soln alkaline, avoiding a large excess; boil for about 5 min., or until any  $\text{NH}_3$  present has been expelled; filter, wash into a 300 cc flask, and make acid with  $\text{HCl}$ , using an excess equivalent to a few cc of 0.1 *N* soln. Boil for 15 min. to expel  $\text{CO}_2$ , cool by placing the flask in cold  $\text{H}_2\text{O}$ , and bring to neutrality by first adding 4 or 5 drops of the methyl red indicator and then the standard  $\text{NaOH}$  soln until the color of the

soln changes from pink to yellow. If the neutral point has been exceeded, or if there is any doubt as to this, restore the pink color by adding a few drops of approximately 0.1 *N* HCl and change the color to yellow again with the minimum quantity of the standard NaOH soln. Add 1–2 g of neutral mannitol (mannite) and a few tenths of a cc of phenolphthalein indicator, 10(d), note the buret reading, and again titrate the soln with the standard NaOH soln until a pink color develops. Add a little more mannitol and if the pink color disappears continue the addition of the standard alkali until it reappears. Repeat this procedure until the addition of mannitol has no further action on the end point. (If the content of boric acid in the soln titrated is low, one addition of mannitol is usually sufficient.) From the volume of the standard alkali required in the titration after the addition of the mannitol, corrected for the volume required when running a blank, calculate the quantity of borax in the sample. 1 cc of 0.1 *N* NaOH soln = 0.0062 g of boric acid, or 0.00503 g of anhydrous borax.

When an acid soln of the sample gives no precipitate upon the addition of a soln of  $\text{CaCl}_2$  and sufficient  $\text{NH}_4\text{OH}$  to give an alkaline reaction, phosphates and Fe and Al salts are absent, and that portion of the determination which involves treatment with  $\text{BaCl}_2$  and  $\text{Ba}(\text{OH})_2$  for the removal of these constituents may be omitted.

(b) *Mixed fertilizers and organic compounds.*—Weigh 5 g of the sample into a 250 cc beaker, add 50 cc of hot  $\text{H}_2\text{O}$ , cover with a watch-glass, digest for 15–20 min. on a water bath, filter, and wash into another beaker of the same capacity. Heat the filtrate to boiling and add 15 cc of 10%  $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$  soln followed without undue loss of time by sufficient powdered  $\text{Ba}(\text{OH})_2$  to give an alkaline reaction as indicated by phenolphthalein; boil for about 5 min. (gently to prevent frothing over), filter, and wash. Or, if preferred, make up to the mark in a volumetric flask and take an aliquot. Evaporate the filtrate or aliquot to dryness in a Pt or porcelain dish and ignite the residue (preferably in a muffle furnace at a temp. just below redness) until the organic matter is completely carbonized. Treat the ignited residue with hot  $\text{H}_2\text{O}$ , make slightly acid with HCl, heat nearly to boiling, make alkaline again with a slight excess of  $\text{Ba}(\text{OH})_2$ , and filter into a 300 cc flask. Acidify with HCl (1+9), using an excess equivalent to a few cc of a 0.1 *N* soln, boil to expel  $\text{CO}_2$ , and titrate as directed under (a).

If the  $\text{Ba}(\text{OH})_2$  has been added only in slight excess there is a tendency for the filtrate to become acid during evaporation with a possible loss of borax. It is important, therefore, that the soln be kept alkaline, by repeated additions of  $\text{Ba}(\text{OH})_2$ , if necessary, until the evaporation is completed.

If the filtrate from the  $\text{BaCl}_2$ - $\text{Ba}(\text{OH})_2$  precipitate is titrated in this determination before the soluble organic matter is destroyed, the end points in the titration will usually be too indefinite to give accurate results. The purpose in evaporating the filtrate and igniting the residue, therefore, is to get rid of soluble organic constituents which interfere with the titration. When the sample contains a relatively high boric acid content, in excess of 0.5%, a smaller sample may be taken and the quantity of organic matter present may then be too small to interfere seriously with the sharpness of the end points during the titration. When such is the case, boil the soln after the addition of the  $\text{Ba}(\text{OH})_2$  until any  $\text{NH}_3$  present has been expelled. Omit evaporating the filtrate from the  $\text{BaCl}_2$ - $\text{Ba}(\text{OH})_2$  precipitate. Add to the filtrate an excess of HCl equivalent to a few cc of a 0.1 *N* soln, boil to expel  $\text{CO}_2$ , and titrate as directed under (a).

#### ACID-SOLUBLE BORIC ACID<sup>14</sup>—OFFICIAL

##### 50

##### APPARATUS

The apparatus<sup>15</sup> consists of two 200 cc round-bottomed flasks, a Liebig condenser, and a 200 cc Erlenmeyer receiving flask. One of the round-bottomed flasks, No. 2,

has a rubber stopper with two holes. Thru one hole passes a glass tube running to the bottom of the flask; thru the other hole passes a short tube leading to the condenser. The other flask, No. 1, is fitted with a perforated rubber stopper and a short bent tube connected by rubber tubing with the long tube in flask No. 2. The whole apparatus is supported by clamps and rings on two stands.

## 51

## DETERMINATION

If the material to be examined is a mixed fertilizer or contains less than the equivalent of 2% of anhydrous borax, weigh 5 g into flask No. 2; if the material contains much more than 2%, use 2 g. Add 5 cc of 85%  $\text{H}_3\text{PO}_4$  and 20 cc of methyl alcohol and connect the flask with the condenser. Add 100 cc of methyl alcohol (at least 95%) to flask No. 1, which is set in a water bath and connected with flask No. 2. Place the receiving flask in position at the end of the condenser and apply sufficient heat to the water bath to keep a steady flow of bubbles of the methyl alcohol passing thru flask No. 2. Also apply some heat to flask No. 2 to keep the volume at about 25 cc. Continue the distillation until 100 cc of distillate is obtained (about 30 min.). When distillation is complete, add 2 or 3 drops of phenolphthalein indicator, 10(d), to the distillate and 5–10 cc of 0.1 N NaOH, or enough to give it a permanent pink color. Stopper the flask, shake well, and connect at once with a condenser by means of a Hopkins or similar bulb. Using a water bath (not a gas burner), distil off the alcohol and save for another determination. Transfer the residue, which should be not less than 10 cc, to a Pt or porcelain dish, using as little  $\text{H}_2\text{O}$  as possible, and evaporate to dryness on a steam or water bath. When dry, ignite below redness, then acidify with a few drops of approximately 1 N HCl, add 20–25 cc of  $\text{H}_2\text{O}$ , and warm for 1–2 min. on a steam bath. Filter into a small flask, thoroly wash, dilute to about 50–75 cc, attach to an air-cooled condenser, and boil gently for a few min. to remove  $\text{CO}_2$ . Add 3 or 4 drops of methyl red indicator, 48(b), and then 0.1 N NaOH until the red color just disappears. Add about 1 g of mannitol, or less if but a small amount of boron is present. (At this point, if boric acid is present, the soln will take on a pinkish color; the depth of color depends on the quantity present, 0.01 or 0.02% usually being sufficient to give the color if the soln has been carefully neutralized with the NaOH soln.) Then add 2 or 3 drops of the phenolphthalein indicator and titrate the soln with the 0.1 N NaOH. Test the reagents by a blank determination. (If the NaOH is free from  $\text{CO}_2$  the blank should not be more than 0.2 cc.) Calculate to boric acid or anhydrous borax as directed under 49(a).

CHLORINE<sup>14</sup>—OFFICIAL

## 52

## REAGENTS

(a) *Standard silver nitrate soln.*—Dissolve about 5 g of pure recrystallized  $\text{AgNO}_3$  in  $\text{H}_2\text{O}$  and dilute to 1 liter. Standardize against pure, dry NaCl and adjust so that 1 cc of the soln  $\approx$  0.001 g of Cl.

(b) *Potassium chromate indicator.*—Dissolve 5 g of  $\text{K}_2\text{CrO}_4$  in 100 cc of  $\text{H}_2\text{O}$ .

## 53

## DETERMINATION

Place 2.5 g of the sample on an 11 cm filter paper and wash with successive portions of boiling  $\text{H}_2\text{O}$  until the washings amount to nearly 250 cc, collecting the filtrate in a 250 cc volumetric flask. Cool, dilute to the mark with  $\text{H}_2\text{O}$ , and mix well. Pipet 50 cc into a 150 cc beaker, add 1 cc of the  $\text{K}_2\text{CrO}_4$  indicator, and titrate with the  $\text{AgNO}_3$  soln until the color produced by  $\text{Ag}_2\text{CrO}_4$  appears as a permanent red.

TOTAL MAGNESIA<sup>17</sup>—TENTATIVE

Weigh 1 g of sample into a 250 cc beaker, and cover. Add 10 cc of HCl and 30 cc of HNO<sub>3</sub> and boil gently for 30 min. Remove the beaker from the source of heat, cool, add 6 cc of H<sub>2</sub>SO<sub>4</sub> (1+1), remove the cover, and evaporate until white fumes appear. Cool slightly, wash down the inside surface of the beaker with a jet of H<sub>2</sub>O, and again evaporate until fumes of H<sub>2</sub>SO<sub>4</sub> appear. Cool, add 10 cc of H<sub>2</sub>O, stir thoroly, and digest on the steam bath for 10–15 min. Remove from the steam bath, add 100 ml of 95% alcohol, stir so that the CaSO<sub>4</sub> is well dispersed thruout the liquid, and allow to stand for 2 hours or longer. Filter by means of suction thru a tight plug of filter paper pulp, using a Gooch crucible, and wash 5 times with 5 cc portions of 95% alcohol containing 1 cc of H<sub>2</sub>SO<sub>4</sub> per 100 cc.

Evaporate the alcoholic filtrate as far as possible on the steam bath. Transfer the soln to a 250 cc Erlenmeyer flask, dilute to 75–100 cc, and add 2 g of citric acid and 10 cc of a 25% soln of (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub>. Add NH<sub>4</sub>OH until the soln is alkaline to litmus and then add 10 cc in excess. Add 5–10 glass beads, tightly stopper the flask, and shake on a shaking machine for at least 1 hour. Allow to stand in a cool place for 4 hours, or preferably overnight. Filter thru a tight paper containing a little paper pulp, and wash with NH<sub>4</sub>OH (5+95), containing 50 g of (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub> per liter, until the precipitate and paper are free from Fe and Al. Pass 25 ml of hot HCl (5+95) thru the paper into the flask, transfer the soln to a 250 cc beaker, and wash the paper and flask thoroly with more of the diluted acid. To the soln in a volume of 50–75 cc and containing no glass beads, add 0.5 ml of a 25% soln of (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub>, cool, and then add NH<sub>4</sub>OH slowly and with stirring until the soln is alkaline to litmus. Stir for a few minutes, then add 3–4 cc of NH<sub>4</sub>OH and allow to stand for 4 hours or overnight. Transfer the precipitate to a small filter or filtering crucible and wash with NH<sub>4</sub>OH (5+95). Ignite slowly in a crucible at a temp. below 900° until the C is burned (preferably in a muffle furnace with pyrometric control), and then at about 1,100° for 1–2 hours. Cool, and weigh.

The residue consists of Mg<sub>2</sub>P<sub>2</sub>O<sub>7</sub> and possibly Mn<sub>2</sub>P<sub>2</sub>O<sub>7</sub> and Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub>. If the alcoholic filtrate is clear, the Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub> will not exceed 0.3 mg and can be neglected. Correct for Mn as follows: Dissolve the residue in 10 cc of H<sub>2</sub>SO<sub>4</sub> (1+9), transfer the soln to a 250 cc Erlenmeyer flask, and add 50 cc of HNO<sub>3</sub> (1+3), 2 cc of sirupy H<sub>3</sub>PO<sub>4</sub> (sp. gr. 1.7), and 0.2 g of KIO<sub>4</sub>. Boil for 15–20 min., cool, and dilute to a convenient volume. In another flask containing the same amounts of the reagents treated in a similar way, match the color by adding a standard soln of KMnO<sub>4</sub>, or compare with a standard soln of KMnO<sub>4</sub> in a colorimeter. From the volume of the soln of permanganate required, or the reading of the colorimeter, calculate the weight of Mn<sub>2</sub>P<sub>2</sub>O<sub>7</sub> in the residue. Subtract this weight from the total weight, and regard the difference as Mg<sub>2</sub>P<sub>2</sub>O<sub>7</sub>, which contains 36.21% of MgO.

ACID-FORMING OR NON-ACID-FORMING QUALITY<sup>18</sup>—TENTATIVE

## REAGENTS

(a) *Methyl red indicator*.—0.2% soln. Dissolve 1 g of the dye in 300 cc of 95% alcohol and dilute to 500 cc with H<sub>2</sub>O.

(b) Na<sub>2</sub>CO<sub>3</sub>-sucrose soln.—Dissolve 106 g of anhydrous Na<sub>2</sub>CO<sub>3</sub> or 286 g of Na<sub>2</sub>CO<sub>3</sub> · H<sub>2</sub>O, and 50 g of sucrose, in H<sub>2</sub>O. Make to a volume of 1 liter. Pipet 10 cc into a 250 cc Erlenmeyer flask, add 30 cc of normal HCl soln carefully, and boil gently for a few minutes to remove CO<sub>2</sub>. Titrate with normal NaOH to the first color change of methyl red indicator. The difference between the volumes of standard acid and base used is the blank for the soln.

56

## DETERMINATION

If the fertilizer mixture, ground as directed under 2, contains less than 30% as the sum of the percentages of total N, available  $P_2O_5$ , and water-soluble potash, weigh 1 g of the mixture into a 100 or 150 cc porcelain or Pyrex glass beaker. If the sum of these percentages is 30 or more, use 0.5 gram, and for salts of Na or K use 0.25 g. With a pipet or buret add 10 cc of the  $Na_2CO_3$ -sucrose soln, and mix thoroly with the fertilizer, except for unmixed nitrate salts. For these, substitute 0.25 g of carbon black for the sucrose. Place in a sand bath to the depth of the mixture in the beaker and evaporate to complete dryness. Place the beakers in a furnace heated to approximately  $250^\circ$ , and raise the temp. gradually to  $500-600^\circ$  (dull red). Hold at this temp. for 1 hour. (It is not necessary that all carbon be removed.) Remove the beaker and allow to cool. Add 50 cc of  $H_2O$ , cover with a watch-glass, and add 30 cc of normal HCl thru the lip of the beaker. After effervescence ceases, place the covered beaker on a hot plate or steam bath and maintain just below the boiling point for 1 hour. Filter the soln thru a disk of filter paper, or a pad of asbestos that has been digested with normal HCl and washed free from acid with  $H_2O$ , using a Gooch crucible and suction. Wash with hot  $H_2O$ . To the entire clear filtrate, approximately 100 cc, add 10 drops of the methyl red indicator, and titrate to the first change in color, orange-pink. (In determining this end point a duplicate soln of the fertilizer ash displaying the maximum acid color for this indicator may be used as a comparison to determine the first change. The titration is conveniently carried out on a white porcelain plate and by using an artificial daylight bulb placed at a convenient angle above and back of the porcelain plate.)

Subtract the cc of normal NaOH used in the titration from the cc of normal HCl added. From the difference subtract algebraically the blank caused by the  $Na_2CO_3$ -sucrose soln. For a 1 g sample multiply the result by 100; 0.5 g sample, by 200; 0.25 g sample, by 400. Positive values represent the excess base in the ash in pounds of  $CaCO_3$  equivalent per ton of fertilizer. Negative values represent excess acidity in the same terms.

Determine total N as directed under 27 or 29. Multiply the percentage of N by 35.7. This is considered to be the acid-forming power of the N in terms of pounds of  $CaCO_3$  equivalent per ton of fertilizer, and is given a negative sign in calculating the net acid-base balance.

Determine the citrate-insoluble  $P_2O_5$  as directed under 16. The percentage found, multiplied by 28.2, is the alkalinity equivalent to 2 of the 3 Ca atoms of  $Ca_3(PO_4)_2$  expressed in terms of pounds of  $CaCO_3$  equivalent per ton of fertilizer. Correct the net balance for the fertilizer for this basicity, assumed to be relatively inactive in the soil, by giving the value a negative sign.

The algebraic sum of the acid-base balance of the ash and the corrections for N and citrate-insoluble  $P_2O_5$  is the net balance of the fertilizer expressed as pounds of  $CaCO_3$  equivalent per ton. If negative, the fertilizer is considered acid forming; if positive, it is considered non-acid forming.

## THOMAS OR BASIC SLAG

57

## MECHANICAL ANALYSIS—OFFICIAL

Proceed as directed under 3, using 10 g of material.

58

## PREPARATION OF SAMPLE—OFFICIAL

Proceed as directed under 2.



## TOTAL PHOSPHORIC ACID

*Gravimetric Method—Official*

59

## PREPARATION OF SOLUTION

Proceed as directed under 8(b), or use HCl alone. In the latter case, measure out the portion for analysis, add 3–5 cc of  $\text{HNO}_3$ , and heat for a few min.

60

## DETERMINATION

Dehydrate an aliquot (20 cc) of the prepared soln by evaporating to dryness on a steam or hot water bath; treat with 5 cc of HCl and 25 cc of hot  $\text{H}_2\text{O}$ , digest in order to complete the soln, and filter off  $\text{SiO}_2$ . From this point proceed as directed under 9. Before precipitating with magnesia mixture, add 5 cc of a 5% Na acetate soln.

61

*Volumetric Method—Official*

Prepare the soln as directed under 8(b) and determine the  $\text{P}_2\text{O}_5$  in an aliquot of this soln as directed under 12, standardizing the solns against a standard phosphate material of approximately the same composition as the sample under examination.

## CITRIC ACID-SOLUBLE PHOSPHORIC ACID

*Gravimetric Method—Official*

62

## PREPARATION OF SOLUTION

Weigh 5 g of the slag, prepared as directed under 2, into a 500 cc Wagner flask containing 5 cc of 95% alcohol. (The neck of the flask should have a width of at least 22 mm, and the graduation marks should be at least 8 cm below the mouth.) Make up to the mark with 2% citric acid soln of a temp. of  $17.5^\circ$ . Fit the flask with a rubber stopper and place at once in a rotary apparatus, shaking the flask for 30 min. at the rate of 30–40 r.p.m. Filter immediately on a dry filter, and analyze the soln at once.

63

## DETERMINATION

To 50 cc of the clear filtrate in a beaker add 100 cc of molybdate soln, 7(a), and place the beaker in a water bath; when the temp. of the contents reaches  $65^\circ$ , remove the beaker and cool to room temp. Filter and wash the yellow precipitate of  $\text{NH}_4$  phosphomolybdate 4 or 5 times with 1%  $\text{HNO}_3$ . Dissolve the precipitate in 100 cc of cold 2%  $\text{NH}_4\text{OH}$ , and nearly neutralize with HCl. Add to the soln dropwise, with continuous stirring, 15 cc of magnesia mixture, 7(c), and proceed as directed under 9.

64

*Volumetric Method—Official*

In an aliquot of the clear soln, prepared as directed under 62, determine the  $\text{P}_2\text{O}_5$  as directed under 12.

## SELECTED REFERENCES

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- <sup>2</sup> Ibid., 15, 65 (1932).
- <sup>3</sup> Ibid., 13, 38 (1930).
- <sup>4</sup> Ibid., 5, 92 (1921); 443 (1922); 6, 384 (1923); 16, 68 (1933); 17, 62 (1934).
- <sup>5</sup> Ibid., 5, 460 (1922).
- <sup>6</sup> Ibid., 19, 107, 194 (1936).

- <sup>7</sup> Chem. Ztg., **16**, 1952 (1892), J. Ind. Eng. Chem., **11**, 306 (1919); **12**, 352 (1920); J. Assoc. Official Agr. Chem., **5**, 450 (1922); **6**, 391 (1923); **15**, 66 (1932).  
<sup>8</sup> J. Ind. Eng. Chem., **11**, 465 (1919).  
<sup>9</sup> J. Assoc. Official Agr. Chem., **13**, 38 (1930).  
<sup>10</sup> Ibid., **18**, 62 (1935); **19**, 68 (1936).  
<sup>11</sup> Ibid., **11**, 34 (1928); **13**, 39 (1930).  
<sup>12</sup> Ibid., **6**, 399 (1923); **7**, 382 (1924); **18**, 63 (1935); **19**, 68 (1936).  
<sup>13</sup> Ibid., **5**, 80 (1921); 327 (1922).  
<sup>14</sup> J. Am. Chem. Soc., **20**, 288 (1898); J. Assoc. Official Agr. Chem., **5**, 88 (1921).  
<sup>15</sup> Leach, Food Inspection and Analysis, 4th ed., 1920, p. 884.  
<sup>16</sup> J. Assoc. Official Agr. Chem., **11**, 34 (1928); **16**, 69 (1933).  
<sup>17</sup> Ibid., **19**, 68 (1936).  
<sup>18</sup> Ibid., **18**, 236 (1935).
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### III. SEWAGE\*

\* See note at bottom of p. xvii.

## IV. AGRICULTURAL LIMING MATERIALS<sup>1</sup>

1

### DIRECTIONS FOR SAMPLING—TENTATIVE

Take a sample that is representative of the lot or shipment and that does not contain a disproportionate quantity of the surface or of any modified, or damaged zone, in the following manner:

(a) *Burnt, or lump lime, in bulk.*—Collect a composite sample of not less than 10 shovelfuls per car, with proportionate quantities from smaller lots, taking each shovelful from a different part of the lot or shipment. Crush immediately to pass a circular opening 1 inch in diameter; mix thoroly and rapidly; quarter down to a 5 lb. sample; and place in a properly labeled, dry, air-tight container.

(b) *Burnt, or lump lime, in barrels.*—Select at random 5 barrels from each lot or shipment of 20 tons or less and 1 additional barrel for each additional 5 tons. Take not less than 10 lbs. from each barrel selected and treat as directed under (a).

(c) *Hydrated lime and ground burnt lime, in bags.*—Select 10 bags from different parts of each lot or shipment of 20 tons or less and 1 additional bag for each additional 5 tons. From each of the bags sampled withdraw a core from top to bottom by means of a sampling tube; mix these portions thoroly and rapidly on heavy sized paper or oilcloth; quarter down to a 2 lb. sample; and place in a properly labeled, dry, air-tight container.

(d) *Ground limestone and ground marl, in bags.*—Proceed as directed under (c).

(e) *Ground limestone, ground burnt lime, and ground marl, in bulk.*—By means of a slotted sampling tube, withdraw samples to full sampler depth from 10 points in the lot or shipment; mix thoroly and rapidly on heavy sized paper or oilcloth; quarter down to a 2 lb. sample; and place in a properly labeled, dry, air-tight container.

2

### PREPARATION OF SAMPLE—TENTATIVE

Grind the sample in a porcelain mortar or porcelain ball mill to pass a 60-mesh sieve, mix thoroly, and preserve in an air-tight container.

### NEUTRALIZING VALUE—TENTATIVE

3

#### REAGENTS

(a) *Sodium hydroxide soln.*—0.25 *N.* Prepare free from carbonates and store in a bottle provided with a siphon tube and with guard tubes containing soda-lime, or other suitable device, to prevent absorption of  $\text{CO}_2$  from the air.

(b) *Nitric acid.*—0.5 *N.* Standardize against (a), using phenolphthalein indicator, II, 10(d).

4

### DETERMINATION

Place 0.5 g of burnt or hydrated lime (1 g of ground limestone or ground marl), prepared as directed under 2, in a 150 cc beaker; add 50 cc of the  $\text{HNO}_3$ , cover the beaker with a watch-glass, and boil for 5 min. Cool, and titrate the excess of acid with the  $\text{NaOH}$  soln, using phenolphthalein indicator. Calculate and report the results as percentage of  $\text{CaO}$  in burnt and hydrated lime and as percentage of  $\text{CaCO}_3$  equivalent for limestone and marl.

### CAUSTIC VALUE<sup>2</sup>—OFFICIAL

5

#### APPARATUS

In the illustration (Fig. 5), *A* is a 500 cc Erlenmeyer flask of Pyrex glass, and *F* is a filter cone packed nearly full with cotton, which is covered to a depth of 2-3 mm

with lightly compacted, macerated filter paper. The filter cone is connected with the syphon tube *B* by means of thick-walled rubber tubing. The receiving flasks *m* and *n* are calibrated to deliver 50 and 100 cc, respectively. *S* is a suction flask.

## DETERMINATION

Transfer a portion of the sample, 2, to a weighing bottle and determine the weight of the weighing bottle plus its contents. (This should be done in an atmosphere of minimum moisture and CO<sub>2</sub> content.) By means of a polished, narrow-pointed spatula that has been calibrated to hold approximately 1.5 g, withdraw the charge to be used and determine its exact weight by difference. Introduce the charge directly into the dry flask (*A*), provided with a tightly fitting rubber stopper.

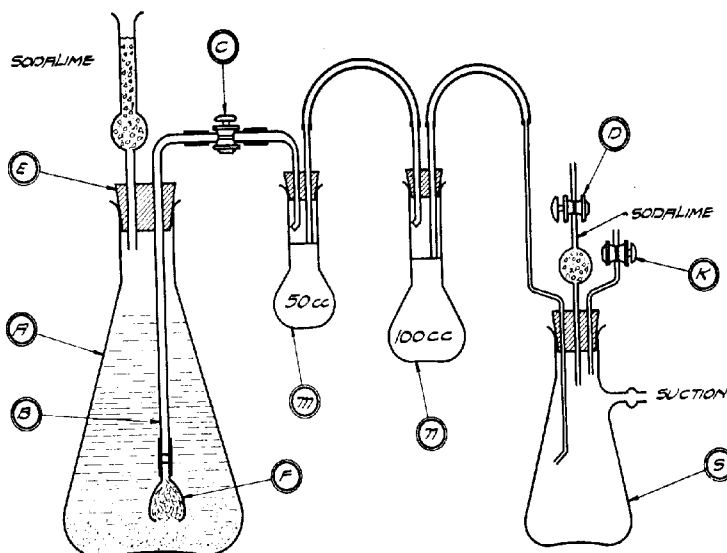


FIG. 5.—APPARATUS FOR AUTOMATIC FILTRATION AND MEASUREMENT OF LIME SOLUTIONS

Prepare a sugar soln *immediately before use* by placing 25 g of granulated sugar in a measuring flask calibrated to deliver 500 cc. Dissolve the sugar with cold CO<sub>2</sub>-free H<sub>2</sub>O and make up to the mark. Holding both the Erlenmeyer flask containing the charge and the flask containing the sugar soln in a slightly inclined position, insert the neck of the sugar soln flask a short distance into the Erlenmeyer flask, and carefully transfer the sugar soln while simultaneously and synchronously agitating both flasks by a rotary motion to prevent granulation of the lime. Stopper the Erlenmeyer flask securely; agitate; and add, if desired, a quantity of clean dry beads. Effect complete soln of uncoated caustic lime by six 1-min. agitations at intervals of 2 or 3 min. Crush any undisintegrated particles of the sample by careful twisting of the stopper after inverting the flask to trap them in the space between the stopper and the neck of the flask. Allow 15 min. further contact between the lime and the sugar soln, and then filter.

Connect the filter cone *F* with the syphon *B* and close stopcock *D*. Connect the

receiving flasks, apply suction, and quickly connect the Erlenmeyer flask (*A*) containing the lime soln with stopper *E*. Open stopcock *C* and filter 25–50 cc of the soln. Close *C* and open *D* to release suction. Remove *m* and replace with another dry flask of the same kind. Close *D*, open *C*, and continue the filtration until both *m* and *n* have been filled at least to the marks. To disconnect the system, close stopcock *C* and press the outlet of flask *m* down gently and then the outlet of flask *n* to remove any excess of liquid above the marks. Permit the intermediate connection to empty, and then open stopcock *D* and remove *m* and *n*. Titrate the first 50 cc, or pilot aliquot, of the filtered soln with 0.5 *N* HCl, using phenolphthalein indicator. Run twice the volume of the 0.5 *N* acid required for this titration into a covered 200 cc beaker, add the second, or 100 cc aliquot, of the filtered soln to this acid and phenolphthalein indicator; and complete the titration.

Calculate the caustic value of the sample by means of the formula:

$$X = \frac{7A}{W}, \text{ in which}$$

*X* = percentage of active CaO;

*A* = cc of 0.5 *N* acid used per 100 cc of lime soln;

*W* = weight of charge.

#### 7 CARBON DIOXIDE—TENTATIVE

Proceed as directed under I, 6, using 5 g of burnt or hydrated lime (1 g of ground limestone or ground marl), prepared as directed under 2. Calculate and report the result as percentage of CaCO<sub>3</sub>.

#### 8 TOTAL CALCIUM OXIDE—TENTATIVE

Place 1 g of burnt or hydrated lime (2 g of ground limestone or ground marl), prepared as directed under 2, in a hard glass beaker of 250 cc capacity; add 25 cc of H<sub>2</sub>O, 10 cc of HCl, and a few drops of HNO<sub>3</sub>; boil for 10 min. and evaporate to dryness. Separate and remove the insoluble matter, SiO<sub>2</sub>, and Fe and Al oxides, as directed under I, 16 and 17. Determine CaO as directed under I, 18.

#### 9 TOTAL MAGNESIUM OXIDE—TENTATIVE

Proceed as directed under I, 19 or 21, using the combined filtrate and washings from the CaO determination under 8.

#### 10 MECHANICAL ANALYSIS OF GROUND LIMESTONE—TENTATIVE

Transfer 100 g of the original material to a set of 10-, 20-, 40-, 60-, 80-, and 100-mesh standardized sieves that comply with the specifications of the Bureau of Standards. Sift, shaking for 5 min. on the 80- and 100-mesh sieves and breaking lumps by means of a soft rubber pestle if the material has a tendency to cake. Weigh the material that is retained on each sieve and that which passes the 100-mesh sieve and express as percentages of the total weight.

#### SELECTED REFERENCES

- <sup>1</sup> J. Assoc. Official Agr. Chem., 7, 252 (1924); U. S. Bur. Standards Circs., 96, 118, 143, 150, 153; Am. Soc. Test. Materials, Tentative Standards, 1923, p. 277.
- <sup>2</sup> Ind. Eng. Chem., 20, 312 (1928); J. Assoc. Official Agr. Chem., 11, 153 (1928); 14, 283 (1931).

### V. AGRICULTURAL DUST\*

\* See note at bottom of p. xvii.

## VI. INSECTICIDES AND FUNGICIDES

1

### PREPARATION OF SAMPLE—OFFICIAL

Thoroughly mix all samples before analysis. Make water-soluble As determinations on samples as received, without further pulverization or drying. In the case of lye, NaCN, or KCN, weigh large quantities in weighing bottles and analyze aliquots of the aqueous solns.

2

### MOISTURE—OFFICIAL

(Applicable to Paris green, London purple, powdered lead arsenate, calcium arsenate, magnesium arsenate, zinc arsenite, and powdered Bordeaux mixture)

Dry 2 g to constant weight at 105–110° and report the loss in weight as moisture.

### TOTAL ARSENIC

#### 1. By Cuprous Chloride Distillation<sup>1</sup>—Official

(Applicable except in the presence of nitrates to the determination of total arsenic in Paris Green, lead arsenate, calcium arsenate, zinc arsenite, magnesium arsenate, and Bordeaux mixture with arsenicals.)

### REAGENTS

(a) *Standard arsenious oxide soln.*—Dissolve 2 g of pure  $As_2O_3$  in a beaker by boiling with about 150–200 cc of  $H_2O$  containing 10 cc of  $H_2SO_4$ , cool, transfer to a 500 cc volumetric flask, and dilute to the mark.

(b) *Standard iodine soln.*—Approximately 0.05 *N*. Mix intimately 6.35 g of pure I with twice this weight of pure KI, dissolve in a small quantity of  $H_2O$ , filter, and dilute the filtrate to 1 liter in a volumetric flask. Standardize against (a) as follows: Pipet 50 cc of the  $As_2O_3$  soln into an Erlenmeyer flask, dilute to the same volume as that of the aliquot used for the titration in the actual determination, neutralize with  $NaHCO_3$ , add 4–5 g in excess, and add the standard I soln from a buret, shaking the flask continuously until the yellow color disappears slowly from the soln. Then add 5 cc of the starch indicator and continue adding the I soln, dropwise, until a permanent blue color is obtained. Calculate the value of the standard I soln in terms of  $As_2O_3$  and  $As_2O_5$ . For the conversion of  $As_2O_3$  to  $As_2O_5$  multiply by 1.1617. Occasionally restandardize the I against the standard  $As_2O_3$  soln.

(c) *Standard bromate soln.*—Dissolve 1.525 g of  $NaBrO_3$  in  $H_2O$  and dilute to 1 liter. One cc of this soln is approximately equal to 0.003 g of  $As_2O_3$ . Standardize against (a) as follows: Pipet 25 cc aliquots of the  $As_2O_3$  soln to 500 cc Erlenmeyer flasks, add 15 cc of  $HCl$ , dilute to 100 cc, heat to 90°, and titrate with the bromate soln, using 5 drops of the methyl orange indicator (f). Do not add the indicator until near the end of the titration, and agitate the liquid continuously in order to avoid local excess of the bromate soln. Add the bromate soln very slowly when approaching the end of the titration; the end point is shown by a change from red to colorless.

(d) *Sodium hydroxide soln.*—Dissolve 400 g of NaOH in  $H_2O$  and dilute to 1 liter.

(e) *Starch indicator.*—Mix about 2 g of finely powdered potato starch with cold  $H_2O$  to a thin paste; add about 200 cc of boiling  $H_2O$ , stirring constantly, and immediately discontinue heating.

(f) *Methyl orange indicator.*—Dissolve 1 g of methyl orange in  $H_2O$  and dilute to 1 liter.

Fig. 6.—The distillation flask is of 500 cc capacity and rests on a metal gauze that fits over a circular hole in a heavy sheet of asbestos board, which in turn extends out far enough to protect the sides of the flask from the direct flame of the burner. The first receiving flask holds 500 cc and contains 40 cc of  $H_2O$ ; the second holds 500 cc and contains 100 cc of  $H_2O$ . The volume in the first flask should not exceed 40 cc, otherwise there may be separated a compound of As that can not readily be redissolved without danger of loss of  $AsCl_3$ . Keep both flasks cool by placing them in a pan thru which  $H_2O$  circulates, or which contains  $H_2O$  and pieces of ice.

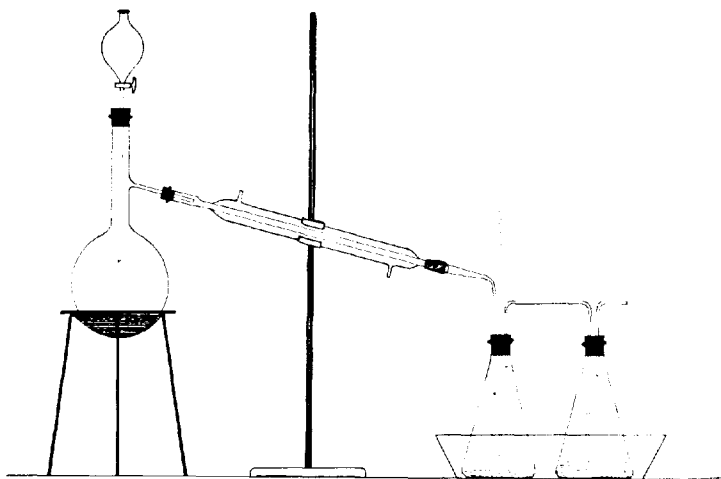


FIG. 6.—APPARATUS FOR DISTILLATION OF ARSENIOS CHLORIDE

Weigh a quantity of the sample containing not more than 0.4 g of As and wash into the distillation flask by means of 100 cc of  $HCl$ . Add 5 g of  $Cu_2Cl_2$  and distil. When the volume in the distillation flask is reduced to about 40 cc add 50 cc more of  $HCl$  by means of the dropping funnel and continue the distillation, repeating the addition of 50 cc portions of  $HCl$  until 200 cc of the acid distillate has passed over. Wash down the condenser and all connecting tubes carefully, transfer these washings and the contents of the Erlenmeyer flasks to a liter volumetric flask, dilute to the mark, and mix thoroly. Titrate the distillate by one of the following procedures:

(a) Pipet a 200 cc aliquot into an Erlenmeyer flask and nearly neutralize with the  $NaOH$  soln, using a few drops of phenolphthalein indicator, II, 10(d), and keeping the soln well cooled. If the neutral point is passed, add  $HCl$  until again slightly acid. Neutralize with  $NaHCO_3$ , add 4–5 g in excess, and add the standard I soln from a buret, shaking the flask continuously until the yellow color disappears slowly from the soln. Then add 5 cc of the starch indicator and continue adding the I soln dropwise until a permanent blue color is obtained.

(b)<sup>2</sup> Pipet a 200 cc aliquot into an Erlenmeyer flask and titrate with the bromate soln as directed under 3(c), beginning with "heat to 90°."

From the number of cc of standard soln used, calculate the percentage of As in the sample. Report as  $\text{As}_2\text{O}_3$  or  $\text{As}_2\text{O}_5$ , according to whether the As is present in the trivalent or pentavalent form. If the condition of the arsenic is unknown, report as As.

### II. By Hydrazine Sulfate Distillation<sup>3</sup>—Official

(Nitrates do not interfere in this method. Applicable to the determination of total arsenic in Paris green, lead arsenate, calcium arsenate, zinc arsenite, magnesium arsenate, and Bordeaux mixture with arsenicals.)

6

#### REAGENTS

*Hydrazine sulfate-sodium bromide soln.*—Dissolve 20 g of  $\text{N}_2\text{H}_4 \cdot \text{H}_2\text{SO}_4$  and 20 g of NaBr in 1 liter of HCl (1+4).

The other reagents and solns are described under 3.

7

#### APPARATUS

The apparatus is described under 4.

8

#### DETERMINATION

Weigh a quantity of the sample containing not more than 0.4 g of As and transfer to the distilling flask. Add 50 cc of the  $\text{N}_2\text{H}_4 \cdot \text{H}_2\text{SO}_4$ -NaBr soln, close the flask with the stopper that carries the funnel tube, and connect the side tube with the condenser. Boil for 2-3 min., add 100 cc of HCl by means of the dropping funnel, and distil until the volume in the distilling flask is reduced to about 40 cc; add 50 cc more of HCl and continue the distillation until the contents of the flask are again reduced to about 40 cc. Wash down the condenser, transfer the contents of the receiving flask to a liter volumetric flask, dilute to volume, and mix thoroly. Titrate the distillate by one of the following procedures:

(a) Proceed as directed under 5(a); or

(b) Pipet a 200 cc aliquot into an Erlenmeyer flask, add 10 cc of HCl, and titrate with the standard bromate soln described under 3(c), beginning with "heat to 90°."

From the number of cc of standard soln used, calculate the percentage of As in the sample. Report as  $\text{As}_2\text{O}_3$  or  $\text{As}_2\text{O}_5$ , according to whether the As is present in the trivalent or pentavalent form. If the condition of the arsenic is unknown, report as As.

### Method III<sup>4</sup>—Tentative

(Applicable in presence of sulfides, sulfites, thiosulfates or large quantities of sulfur.)

9

#### REAGENTS

*Sodium thiosulfate soln.*—Approximately 0.05 N. Dissolve 13 g of crystallized  $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$  in  $\text{H}_2\text{O}$  and dilute to 1 liter.

The other reagents and solns are described under 3. The apparatus is described under 4.

10

#### DETERMINATION

Weigh 2 g of the sample and transfer to the distilling flask. Add a soln of 5-8 g of  $\text{Cu}_2\text{Cl}_2$  in 100 cc of HCl and shake to bring the sample completely in contact with the acid soln and to expel  $\text{H}_2\text{S}$ . When the reaction has ceased, close the flask, connect with the condenser, and distil as directed under 5 until 200 cc of the acid dis-



tillate has passed over. Make the distillate to volume in a liter flask, mix thoroly, and transfer a 200 cc aliquot to a 400 cc Pyrex beaker or porcelain casserole. Add 10 cc of  $\text{HNO}_3$  and 5 cc of  $\text{H}_2\text{SO}_4$ , evaporate to a sirupy consistency on a steam bath, and then heat on a hot plate until the white fumes of  $\text{H}_2\text{SO}_4$  appear. Cool, and wash into a 500 cc Erlenmeyer flask. If the quantity of  $\text{H}_2\text{SO}_4$  is appreciably lessened by fuming, add sufficient to make the total quantity of  $\text{H}_2\text{SO}_4$  approximately 5 cc. Dilute to 100–150 cc, add 1.5 g of KI, and boil until the volume is reduced to about 40 cc. Cool the soln under running  $\text{H}_2\text{O}$ ; dilute to 100–150 cc; and add the  $\text{Na}_2\text{S}_2\text{O}_3$  soln, 9, dropwise until the I color is just removed. Nearly neutralize the  $\text{H}_2\text{SO}_4$  with the NaOH soln, 3(d), finish the neutralization with  $\text{NaHCO}_3$ , add 4–5 g in excess, and titrate with the standard I soln as directed under 3(b). From the number of cc of standard soln used calculate the percentage of As in the sample. Report as  $\text{As}_2\text{O}_3$  or  $\text{As}_4\text{O}_6$  according to whether the As is present in the trivalent or pentavalent form. If the condition of the arsenic is unknown, report as As.

#### WATER-SOLUBLE ARSENIC—OFFICIAL

(Applicable to the determination of water-soluble arsenic in lead arsenate, calcium arsenate, zinc arsenite, magnesium arsenate, and Bordeaux mixture with arsenicals.)

#### 11

##### REAGENTS

The reagents and solns are described under 3 and 9.

#### 12

##### DETERMINATION

To 2 g of the original sample if a powder, or 4 g if a paste, in a liter Florence flask, add 1 liter of recently boiled  $\text{H}_2\text{O}$  that has been cooled to  $32^\circ$ . Stopper the flask and place in a water bath kept at  $32^\circ$  by means of a thermostat. Digest for 24 hours, shaking hourly for 8 hours during this period. Filter thru a dry filter, transfer 250–500 cc of the clear filtrate to an Erlenmeyer flask, add 3 cc of  $\text{H}_2\text{SO}_4$ , and evaporate on a hot plate. When the volume reaches approximately 100 cc add 1 g of KI, and continue the boiling until the volume is about 40 cc. Cool, dilute to about 200 cc, and add the  $\text{Na}_2\text{S}_2\text{O}_3$  soln, 9, dropwise until the I color is exactly removed. (Avoid the use of starch indicator at this point.) Neutralize with  $\text{NaHCO}_3$ , add 4–5 g in excess, titrate with the standard I soln until the yellow color disappears slowly, add 5 cc of the starch indicator, and continue the titration to a permanent blue color. Make correction for the quantity of standard I soln necessary to produce the same color, using the same reagents and volume. From the number of cc of standard I soln used calculate the percentage of water-soluble As in the sample.

#### 13

##### LEAD OXIDE—OFFICIAL\*

Weigh 1 g of the powdered sample and transfer to a beaker. Add 5 cc of  $\text{HBr}$  (approximately 1.38 sp. gr.) and 15 cc of  $\text{HCl}$ , and evaporate to dryness to remove As. Repeat the treatment, add 20 cc more of the  $\text{HCl}$ , and again evaporate to dryness. Add to the residue 25 cc of the 2 N  $\text{HCl}$ , heat to boiling, filter immediately to remove  $\text{SiO}_2$ , and wash with boiling  $\text{H}_2\text{O}$  to a volume of 125 cc. See that all  $\text{PbCl}_2$  is in soln before filtering; if it will not dissolve completely in 25 cc of 2 N acid, add 25 cc additional and dilute the filtrate to 250 cc volume. Pass in  $\text{H}_2\text{S}$  until the precipitation is complete. Filter, and wash the precipitate thoroly with 0.5 N  $\text{HCl}$  saturated with  $\text{H}_2\text{S}$ . Save the filtrate and washings for the determination of zinc.

\* Applicable to such preparations as Bordeaux-lead arsenate, Bordeaux-zinc arsenite, Bordeaux-Paris green, and Bordeaux-calcium arsenate.

## INSECTICIDES AND FUNGICIDES

Transfer the filter paper containing the sulfides of Pb and Cu to a 400 cc Pyrex beaker and completely oxidize all organic matter by heating on a steam bath with 4 cc of  $\text{H}_2\text{SO}_4$  and about 20 cc of fuming  $\text{HNO}_3$  in a covered beaker. Evaporate on the steam bath and then completely remove  $\text{HNO}_3$  by heating on a hot plate until copious evolution of the white fumes of  $\text{H}_2\text{SO}_4$  occurs. Cool, add 2-3 cc of  $\text{H}_2\text{O}$ , and again heat to fuming. Cool, add 50 cc of  $\text{H}_2\text{O}$  and 100 cc of 95% alcohol, and let stand several hours (preferably overnight). Filter thru a Gooch crucible, previously washed with  $\text{H}_2\text{O}$ , with acidified alcohol (100 parts of  $\text{H}_2\text{O}$ , 200 parts of 95% alcohol, and 3 parts of  $\text{H}_2\text{SO}_4$ ) and with 95% alcohol, and then dried at  $200^\circ$ . Wash the precipitate of  $\text{PbSO}_4$  in the crucible about 10 times with the acidified alcohol, and then with 95% alcohol, to remove  $\text{H}_2\text{SO}_4$ . Dry at  $200^\circ$  to constant weight, keeping the crucible covered to prevent loss from spattering. From the weight of  $\text{PbSO}_4$ , calculate the percentage of PbO in the sample, using the factor 0.7360.

### COPPER\*

14

#### *Electrolytic Method—Official*

Evaporate the filtrate and washings from the  $\text{PbSO}_4$  precipitation, 13, to fuming; add a few cc of fuming  $\text{HNO}_3$  to destroy organic matter; and continue the evaporation until about 3 cc remains. Take up with about 100 cc of  $\text{H}_2\text{O}$ , add 1 cc of  $\text{HNO}_3$ , and filter, if necessary. Wash into a weighed 150 cc Pt dish and electrolyze, using a rotating anode and a current of about 3 ampercs. (In lieu of the Pt dish a 150 cc beaker and a weighed gauze cathode may be used.) After all the Cu has been deposited (requiring about 30 min.) and while the current is still flowing, wash the deposit with  $\text{H}_2\text{O}$  by siphoning. Then interrupt the current, rinse the cathode with alcohol, dry for a few moments in an oven, and weigh. Calculate the percentage of Cu in the sample.

15

#### *Thiosulfate Volumetric Method—Official*

Proceed as directed under 14 to the point at which the filtrate and washings from the  $\text{PbSO}_4$  precipitation have been treated with fuming  $\text{HNO}_3$  and evaporated to a volume of about 3 cc. Take up in about 50 cc of  $\text{H}_2\text{O}$ , add  $\text{NH}_4\text{OH}$  in excess, and boil until the excess of  $\text{NH}_3$  is expelled, as shown by a change of color in the liquid and a partial precipitation. Then add 3-4 cc of 80% acetic acid, boil 1-2 min., cool, add 10 cc of a 30% KI soln, and titrate with standard thiosulfate soln (XXXIV, 39) until the brown color becomes faint. Then add starch indicator, 3(e), and continue the titration cautiously until the blue color due to free I has entirely vanished. From the number of cc of standard thiosulfate soln used calculate the percentage of Cu in the sample.

### ZINC OXIDE\*—OFFICIAL\*

16

#### REAGENT

*Mercury-thiocyanate soln.*—Dissolve 27 g of  $\text{HgCl}_2$  and 30 g of  $\text{NH}_4\text{SCN}$  in  $\text{H}_2\text{O}$  and dilute to 1 liter.

17

#### DETERMINATION

Concentrate the filtrate and washings from the sulfide precipitation, 13, by gentle boiling to about 50 cc, and continue the evaporation on a steam bath to dryness. Dissolve the residue in 100 cc of  $\text{H}_2\text{O}$  containing 5 cc of  $\text{HCl}$ , and add 35-40 cc of the Hg-thiocyanate reagent with vigorous stirring. Allow to stand at least an hour

\* Applicable to such preparations as Bordeaux-lead arsenate, Bordeaux-zinc arsenite, Bordeaux-Paris green, and Bordeaux-calcium arsenate.

with occasional stirring. Filter thru a weighed Gooch crucible, wash with  $\text{H}_2\text{O}$  containing 20 cc of the Hg-thiocyanate reagent per liter, and dry to constant weight at  $105^\circ$ . From this weight calculate the percentage of ZnO in the sample, using the factor 0.16332.

Some Fe is usually present and during the Zn determination should be in the ferrous condition. In making the sulfide precipitation the  $\text{H}_2\text{S}$  should be passed into the soln for a sufficient length of time to reduce the Fe, as well as to precipitate the Cu and Pb. The Zn-Hg-thiocyanate precipitate normally is white, and the occluded ferric thiocyanate should not give it more than a faint pink color.

#### FLUORINE

##### *Distillation Method*<sup>7</sup>—*Tentative*

#### 18

##### REAGENTS

(a) *Sodium alizarin sulfonate soln.*—Dissolve 0.1 g of sodium alizarin sulfonate in 200 cc of  $\text{H}_2\text{O}$ , and filter if necessary.

(b) *Standard thorium nitrate soln.*—Approximately 0.02 *N*. Dissolve 3.4917 g of crystallized  $\text{Th}(\text{NO}_3)_4 \cdot 12\text{H}_2\text{O}$  in  $\text{H}_2\text{O}$  and dilute to 1 liter. Standardize as follows:

Transfer 10 cc of 0.02 *N* NaF soln to a small porcelain casserole (or a tall-form 50 cc beaker over a white surface), add 4 drops of the alizarin indicator and 20 cc of neutral 95% alcohol. If the soln is not yellow after the addition of the indicator, add dropwise  $\text{HCl}$  (1 + 50) until the yellow color just appears and then add 3 drops in excess. Titrate to a faint permanent pink with the thorium nitrate. (The soln shades from yellow to pink as the end point is approached, and the use of a reference soln of the indicator with a slight pink color insures more accurate results.) Because the pink color develops slowly as the end point is approached, use care to avoid over titration. Calculate the value of the thorium nitrate in terms of F per cc (the aliquot of NaF soln used for standardization should contain approximately the same quantity of F and have the same volume as the aliquot of the sample to be titrated).

#### 19

##### DETERMINATION

(a) *In water-soluble fluorine compounds (interfering elements absent).*—Dissolve about 0.2 g of the salt in  $\text{H}_2\text{O}$  and dilute to 200 cc. Transfer a 10 cc aliquot of this soln to a small porcelain casserole (or a small tall-form beaker over a white surface), add 4 drops of indicator and 20 cc of neutral 95% alcohol. Add  $\text{HCl}$  (1 + 50) dropwise until the pink color is exactly discharged and then add 3 drops in excess. Titrate with the standard thorium nitrate soln to a reappearance of the pink color as directed under 18 b). From this titration calculate the percentage of F in the sample.

(b) *In water-insoluble fluorine compounds, water-soluble fluorine compounds with interfering elements present (organic matter absent or present only in small quantity).*—Weigh a quantity of the sample equivalent to 0.07–0.10 g of F into a 50 or 100 cc Pyrex Claissen-type distillation flask (50 cc flask preferred). Add 8–12 glass beads, 10 cc of  $\text{H}_2\text{O}$  and 6–7 cc of 60% perchloric acid, or 6–7 cc  $\text{H}_2\text{SO}_4$  if there is danger of an explosive mixture. Do not use perchloric acid on samples containing the slightest trace of sulfur or organic matter. Support the flask on an asbestos mat having a circular opening sufficiently large to expose the lower third of the flask to the flame. Close the flask with a 2-holed rubber stopper through which passes a thermometer and a capillary tube, both of which should extend into the liquid. Connect a 50 cc dropping funnel with the capillary tube, so that  $\text{H}_2\text{O}$  may be added

during the distillation, and connect the side-neck of the distilling flask with a condenser. Collect the distillate in a 200 cc volumetric flask. Heat slowly until an initial boiling point of 110–112° is reached. Continue the distillation until the boiling point reaches 135° and hold at approximately that temp. (must not exceed 140°) by allowing H<sub>2</sub>O to enter slowly from the dropping funnel until the distillate measures 150 cc. Make the soln to volume, transfer a 10 cc aliquot to a small porcelain casserole (or a small tall-form beaker over a white surface), add 4 drops of indicator and 1% NaOH soln dropwise until the color of the indicator appears, exactly discharge the color with HCl (1+50), and add 3 drops in excess. Add 20 cc of alcohol and titrate with the thorium nitrate soln to a reappearance of the pink color. From this titration calculate the percentage of F in the sample.

NOTE.—Procedure (b) must be used for samples containing ions that form a precipitate or non-dissociated salt with F or with Th (e.g., Ca, Ba, Fe, Al, PO<sub>4</sub>, etc.).

(c) *In presence of much organic matter.*—Weigh a quantity of the sample not exceeding 5 g and containing 0.1 g or less of F, in a 50 cc Pt dish, and add a quantity of Ca(OH)<sub>2</sub> containing approximately 1 g of CaO. Add small quantities of H<sub>2</sub>O with stirring until the charge is thoroly moistened and intimately mixed. Dry completely in a hot air oven at 105°, and then ignite gently over a flame until the charge is just charred, and complete the ignition in a muffle furnace heated to dull redness. Transfer the ash to a 100 cc Pyrex Claissen-type distillation flask and proceed as directed in (b) except to use 10 cc of H<sub>2</sub>SO<sub>4</sub>.

NOTES.—An ordinary side-neck flask can be used instead of the Claissen type flask for the distillation, but it is preferable to bend the side-neck so that it is inclined upward and then back again to its original angle with the flask. At the end of the titrations the soln should contain 50–60% by volume of alcohol. When lime is used as a fixative for F, a correction should be made for any F contained in it.

## PARIS GREEN

### 20 MOISTURE—OFFICIAL

Proceed as directed under 2.

### 21 TOTAL ARSENIC - OFFICIAL

Proceed as directed under 5 or 8.

### TOTAL ARSENIOS OXIDE

(The following methods determine only the As present in the trivalent form (As<sub>2</sub>O<sub>3</sub>). They also determine any Sb that may be present in the trivalent form (Sb<sub>2</sub>O<sub>3</sub>). Ferrous and cuprous salts vitiate the results.)

#### *Method 1<sup>s</sup>—Official*

### 22 REAGENTS

*Ammonium chloride soln.*—Dissolve 250 g of NH<sub>4</sub>Cl in H<sub>2</sub>O and dilute to 1 liter. The other reagents and solns are described under 3.

### 23 DETERMINATION

Weigh 0.3 g of the sample and wash into an Erlenmeyer flask with 10–15 cc of HCl (1+4) or 10–15 cc of H<sub>2</sub>SO<sub>4</sub> (1+4), followed by about 100 cc of H<sub>2</sub>O, and heat on a steam bath only so long as is necessary to complete soln, at a temp. not exceeding 90°. (If H<sub>2</sub>SO<sub>4</sub> is used the soln may be heated to boiling.) Cool, neutralize with NaHCO<sub>3</sub>, add 4–5 g in excess, and then add sufficient NH<sub>4</sub>Cl soln to dissolve the

precipitated Cu. Dilute somewhat and titrate as directed under 3(b). Make correction for the quantity of I soln necessary to produce a blue color with starch in the presence of Cu (using an equivalent weight of  $\text{CuSO}_4$ ). From the corrected number of cc standard I soln used calculate the percentage of  $\text{As}_2\text{O}_3$  in the sample.

24

*Method II<sup>9</sup>—Official*

Weigh 1.5 g of the sample and wash into a 250 cc volumetric flask with 100 cc of HCl (1+4), heating to a maximum of 90°, if necessary, to secure complete soln of the sample. Cool, and make to volume.

(a) Transfer a 50 cc aliquot to a 500 cc Erlenmeyer flask, add 10 cc of HCl, heat to 90°, and titrate with the standard bromate soln as directed under 3(c), beginning with "titrate with the bromate soln." Or,

(b) Proceed as directed under (a) but make the titration without heating the soln.

From the number of cc of bromate soln used calculate the percentage of  $\text{As}_2\text{O}_3$  in the sample.

SODIUM ACETATE-SOLUBLE ARSENIOUS OXIDE<sup>10</sup>—TENTATIVE

25

## REAGENTS

(a) *Sodium acetate soln.*—12.5 g of the crystallized salt ( $\text{CH}_3\text{COONa} \cdot 3\text{H}_2\text{O}$ ) in each 25 cc.

The other reagents and solns are described under 3.

26

## DETERMINATION

Place 1 g of the sample in a 100 cc volumetric flask and boil for 5 min. with 25 cc of the Na acetate soln. Dilute to the mark, shake, and pass thru a dry filter paper. Titrate an aliquot of this filtrate as directed under 3(b). Calculate the quantity of  $\text{As}_2\text{O}_3$  present and express the results as percentage of Na-acetate-soluble  $\text{As}_2\text{O}_3$ .

27

## WATER-SOLUBLE ARSENIOUS OXIDE—OFFICIAL

To 1 g of the sample in a liter Florence flask add 1 liter of recently boiled  $\text{H}_2\text{O}$  that has been cooled to 32°. Stopper the flask and place in a water bath kept at 32° by means of a thermostat. Digest for 24 hours, shaking hourly for 8 hours during this period. Filter thru a dry filter and transfer 250 cc of the filtrate to an Erlenmeyer flask; add 4–5 g of  $\text{NaHCO}_3$  and titrate with the I soln, 3(b), to a permanent blue color, using starch indicator, 3(e). Correct for the quantity of the I soln necessary to produce the same color, using the same reagents and volume. Calculate the quantity of  $\text{As}_2\text{O}_3$  present and express the results as percentage of water-soluble  $\text{As}_2\text{O}_3$ .

## TOTAL COPPER OXIDE

28

*Electrolytic Method—Official*

Treat 2 g of the sample in a beaker with 100 cc of  $\text{H}_2\text{O}$  and about 2 g of NaOH and boil thoroly until all the Cu is precipitated as  $\text{Cu}_2\text{O}$ . Filter, wash well with hot  $\text{H}_2\text{O}$ , dissolve the precipitate in hot  $\text{HNO}_3$  (1+4), cool, transfer to a 250 cc volumetric flask, and dilute to the mark. Electrolyze an aliquot of 50 or 100 cc, as directed under 14. Calculate to percentage of CuO.

29

*Volumetric Thiosulfate Method<sup>11</sup>—Official*

Determine Cu in an aliquot of the  $\text{HNO}_3$  soln of  $\text{Cu}_2\text{O}$ , 28, by titrating with standard thiosulfate soln as directed under 15, and calculate to percentage of CuO.

**LEAD ARSENATE**

30

**MOISTURE—OFFICIAL**

(a) *Powder*.—Dry 2 g to constant weight at 105–110° and report the loss in weight as moisture.

(b) *Paste*.—Proceed as directed under (a), using 50 g. Grind the dry sample to a fine powder, mix well, transfer a small portion to a sample bottle, and again dry for 1–2 hours at 105–110°. Use this anhydrous material for the determination of total PbO and total As.

**TOTAL ARSENIC**

31

*Method I—Official*

Proceed as directed under 5 or 8.

32

*Method II<sup>12</sup>—Official*

(Not applicable in the presence of antimony.)

Dissolve 1 g of the powdered sample with  $\text{HNO}_3$  (1+4) in a porcelain casserole or evaporating dish, add 5 cc of  $\text{H}_2\text{SO}_4$ , and heat on a hot plate to copious evolution of white fumes. Cool, add a little  $\text{H}_2\text{O}$ , and again evaporate until the appearance of white fumes to assure removal of the last trace of  $\text{HNO}_3$ . Wash into a 200 cc volumetric flask with  $\text{H}_2\text{O}$ , cool, dilute to volume, and filter thru a dry filter. Transfer 100 cc of the filtrate to an Erlenmeyer flask and proceed as directed under 12, beginning with “add 1 gram of KI.” From the number of cc of standard I soln used calculate the percentage of total As in the sample in terms of  $\text{As}_2\text{O}_5$ .

33

**TOTAL ARSENIOS OXIDE<sup>13</sup>—OFFICIAL**

Weigh 2 g of the powdered sample and transfer to a 200 cc volumetric flask, add 100 cc of  $\text{H}_2\text{SO}_4$  (1+6), and boil for 30 min. Cool, dilute to volume, shake thoroly, and filter thru a dry filter. Nearly neutralize 100 cc of the filtrate with NaOH soln, 3(d), using a few drops of phenolphthalein indicator, II, 10(d). If the neutral point is passed, make acid again with the dilute  $\text{H}_2\text{SO}_4$ . Continue as directed under 3(b), beginning “neutralize with  $\text{NaHCO}_3$ .” From the number of cc of standard I soln used calculate the percentage of  $\text{As}_2\text{O}_5$ .

**TOTAL ARSENIC OXIDE<sup>14</sup>—TENTATIVE**

34

**REAGENTS**

(a) *Potassium iodide soln.*—Dissolve 20 g of KI in  $\text{H}_2\text{O}$  and dilute to 100 cc.

(b) *Standard thiosulfate soln.*—Prepare an approximately 0.05 N soln as follows: Dissolve 13 g of  $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$  in recently boiled and cooled  $\text{H}_2\text{O}$ , filter, and dilute to 1 liter with recently boiled and cooled  $\text{H}_2\text{O}$ . Standardize as follows:

Dissolve about 0.7 g of  $\text{PbHAsO}_4$  in 50 cc of HCl in an Erlenmeyer flask. If necessary to effect soln, heat on a steam bath, keeping the flask covered with a watch-glass to prevent evaporation of the acid. Cool to 20–25°, add 10 cc of the KI soln, (a), and 50 cc (or more if necessary to produce a clear soln) of  $\text{NH}_4\text{Cl}$  soln, 22, and immediately titrate the liberated I with the standard thiosulfate. When the color becomes a faint yellow, dilute with about 150 cc of  $\text{H}_2\text{O}$  and continue the titration carefully, dropwise, until colorless, using starch indicator, 3(e), near the end point. From the weight of  $\text{PbHAsO}_4$  and the number of cc of  $\text{Na}_2\text{S}_2\text{O}_3$  soln used calculate the value of the latter in terms of  $\text{As}_2\text{O}_5$ . ( $\text{As}_2\text{O}_5$  in  $\text{PbHAsO}_4 = 33.11\%$ .)

Prepare pure  $\text{PbHAsO}_4$  by pouring a soln of  $\text{Pb}(\text{NO}_3)_2$  into a soln of  $\text{KH}_2\text{AsO}_4$ , which should be in excess. Collect the precipitate by filtration, dissolve it in the

smallest possible quantity of boiling  $\text{HNO}_3$  (1+4), and pour the soln into a large quantity of  $\text{H}_2\text{O}$  (approximately 50–100 cc of the  $\text{HNO}_3$  soln in 2–3 liters of  $\text{H}_2\text{O}$ ). Collect the precipitate by filtration and dry at  $110^\circ$ .

35

## DETERMINATION

Weigh 0.5 g of the powdered sample and transfer to an Erlenmeyer flask. Add 25–30 cc of  $\text{HCl}$  and evaporate to dryness on a steam bath. Then add 50 cc of  $\text{HCl}$  and proceed as directed under 34(b), beginning with "If necessary to effect soln, heat on a steam bath." From the number of cc of standard thiosulfate soln used calculate the percentage of  $\text{As}_2\text{O}_3$ .

36

## WATER-SOLUBLE ARSENIC—OFFICIAL

Proceed as directed under 12, and calculate results as  $\text{As}_2\text{O}_3$ .

## TOTAL LEAD OXIDE

37

*Method 1<sup>15</sup>—Official*

Heat in a 600 cc beaker on a hot plate, 0.5 g of the powdered sample and about 25 cc of  $\text{HNO}_3$  (1+4). Remove any insoluble residue by filtration. Dilute to at least 400 cc; heat nearly to boiling; and add  $\text{NH}_4\text{OH}$  to slight precipitation, then  $\text{HNO}_3$  (1+9) to redissolve the precipitate, adding 1–2 cc in excess. Pipet into this soln, kept almost boiling, 50 cc of a hot 10%  $\text{K}_2\text{CrO}_4$  soln, stirring constantly. Decant while hot thru a weighed Gooch crucible, previously heated to  $140$ – $150^\circ$ , and wash several times by decantation and then on the filter with boiling  $\text{H}_2\text{O}$  until the washings are colorless. Dry the  $\text{PbCrO}_4$  at  $140$ – $150^\circ$  to constant weight. From the weight of the  $\text{PbCrO}_4$  calculate the percentage of  $\text{PbO}$  in the sample, using the factor 0.6906.

The  $\text{PbCrO}_4$  precipitate may contain a small quantity of  $\text{PbHAsO}_4$ , which will cause slightly high results, but this error rarely amounts to more than 0.1–0.2%.

38

*Method 1<sup>16</sup>—Official*

(Not applicable in the presence of calcium.)

Heat in a porcelain evaporating dish or casserole on a hot plate, 0.5 g of the powdered sample and about 25 cc of  $\text{HNO}_3$  (1+4). Remove any insoluble residue by filtration. Add 3 cc of  $\text{H}_2\text{SO}_4$  and evaporate on a hot plate until the appearance of white fumes. Cool, add a few cc of  $\text{H}_2\text{O}$  (to decompose any nitro-sulfuric acid formed), and again heat to fuming. Proceed as directed under 13, beginning with "Cool, add 50 cc of  $\text{H}_2\text{O}$  and 100 cc of 95% alcohol."

## CALCIUM ARSENATE

39

## MOISTURE—OFFICIAL

Proceed as directed under 2.

40

## TOTAL ARSENIC

Proceed as directed under 5 or 8.

41

TOTAL ARSENIOS OXIDE<sup>17</sup>—OFFICIAL

(a) (Not applicable in the presence of nitrates.)

Weigh 1 g of the sample, transfer to a 500 cc Erlenmeyer flask, and dissolve in 100 cc of  $\text{HCl}$  (1+3). Heat to  $90^\circ$  and titrate with the standard bromate soln, 3(c),

using 5 drops of the methyl orange indicator, 3(f). From the number of cc of standard bromate soln used calculate the percentage of  $\text{As}_2\text{O}_3$  in the sample.

(b) (Applicable in the presence of small quantities of nitrates.)

Proceed as directed under (a) except to make the titration at room temp.

42

#### WATER-SOLUBLE ARSENIC—OFFICIAL

Proceed as directed under 12, and calculate results as  $\text{As}_2\text{O}_5$ .

#### TOTAL CALCIUM OXIDE

##### Method I<sup>17</sup>—Official

43

#### REAGENTS

(a) *Ammonium oxalate soln.*—Dissolve 40 g of  $(\text{NH}_4)_2\text{C}_2\text{O}_4 \cdot \text{H}_2\text{O}$  in 1 liter of  $\text{H}_2\text{O}$ .

(b) *Standard potassium permanganate soln.*—Dissolve 3.161 g of  $\text{KMnO}_4$  in freshly distilled  $\text{H}_2\text{O}$  and dilute to 1 liter. Filter thru asbestos in a Gooch crucible and allow to stand for several days in a dark place. To standardize, dissolve 0.25 g of pure  $\text{Na}_2\text{C}_2\text{O}_4$  in  $\text{H}_2\text{O}$ , add 25 cc of  $\text{H}_2\text{SO}_4$  (1+4), dilute to 200 cc, heat to about  $70^\circ$ , and titrate with the  $\text{KMnO}_4$  soln until the soln assumes a faint pink color. From this titration calculate the strength of the  $\text{KMnO}_4$  soln, which should be approximately 0.1 N.

44

#### DETERMINATION

Dissolve 2 g of the sample in 80 cc of acetic acid (1+3), transfer to a 200 cc volumetric flask, dilute to volume, and filter thru a dry filter. Transfer a 50 cc aliquot to a beaker, dilute to about 200 cc, heat to boiling, and precipitate the Ca with the  $(\text{NH}_4)_2\text{C}_2\text{O}_4$  soln. Allow the beaker to stand 3 hours on a steam bath, filter the soln and wash the precipitate with hot  $\text{H}_2\text{O}$ . Dissolve the precipitate in 200 cc of  $\text{H}_2\text{O}$  containing 25 cc of  $\text{H}_2\text{SO}_4$  (1+4), heat to about  $70^\circ$ , and titrate with the standard  $\text{KMnO}_4$  soln. From the number of cc of standard  $\text{KMnO}_4$  soln used calculate the percentage of CaO.

45

##### Method II<sup>17</sup>—Official

(Not applicable in the presence of lead.)

Weigh 2 g of the sample; transfer to a beaker, add 5 cc of  $\text{HBr}$  (approximately 1.38 sp. gr.) and 15 cc of  $\text{HCl}$ , and evaporate to dryness under a hood to remove As. Repeat the treatment, add 20 cc of  $\text{HCl}$ , and again evaporate to dryness. Take up with  $\text{H}_2\text{O}$  and a little  $\text{HCl}$ , filter into a 200 cc volumetric flask, wash, and dilute to volume. Transfer a 50 cc aliquot to a beaker, add 10 cc of  $\text{HCl}$  and a few drops of  $\text{HNO}_3$ , boil, and make slightly alkaline with  $\text{NH}_4\text{OH}$ . Let stand a few min. and filter. Dissolve the precipitate in  $\text{HCl}$  (1+4), reprecipitate, filter thru the same paper, and wash with hot  $\text{H}_2\text{O}$ . To the combined filtrates and washings add 20 cc of acetic acid (1+3) and adjust the volume to about 200 cc. Heat to boiling, precipitate with the  $(\text{NH}_4)_2\text{C}_2\text{O}_4$  soln, 43(a), and allow to stand for 3 hours on a steam bath. Filter and wash with hot  $\text{H}_2\text{O}$ . Ignite, and weigh as  $\text{CaO}$ ; or dissolve the precipitate in 200 cc of  $\text{H}_2\text{O}$  containing 25 cc of  $\text{H}_2\text{SO}_4$  (1+4), heat to about  $70^\circ$ , and titrate with the  $\text{KMnO}_4$  soln, 43(b). From the weight of  $\text{CaO}$  or the number of cc of standard  $\text{KMnO}_4$  soln used calculate the percentage of  $\text{CaO}$ .

#### MAGNESIUM ARSENATE

46

#### MOISTURE—OFFICIAL

Proceed as directed under 2.



**47 TOTAL ARSENIC OFFICIAL**

Proceed as directed under 5 or 8.

**48 TOTAL ARSENIOS OXIDE—OFFICIAL**

Proceed as directed under 33.

**49 WATER-SOLUBLE ARSENIC—OFFICIAL**

Proceed as directed under 12, and calculate the results as  $\text{As}_2\text{O}_3$ .

**ZINC ARSENITE****50 MOISTURE—OFFICIAL**

Proceed as directed under 2.

**51 TOTAL ARSENIC—OFFICIAL**

Proceed as directed under 5 or 8, and calculate results as  $\text{As}_2\text{O}_3$ .

**TOTAL ARSENIOS OXIDE****52 Method I<sup>7</sup>—Official**

(a) Weigh 2 g of the sample and transfer to a beaker. Dissolve in 80 cc of HCl (1+4), wash into a 200 cc volumetric flask, and dilute to volume. Thoroughly mix the soln and filter thru a dry filter. Transfer a 25 cc aliquot to a 500 cc Erlenmeyer flask, add 20 cc of HCl, and dilute to 100 cc. Heat to 90° and titrate with the standard bromate soln, 3(c). Or,

(b) Proceed as directed under (a) without heating the soln.

**53 Method II—Official**

Proceed as directed under 23.

**54 WATER-SOLUBLE ARSENIC OFFICIAL**

Proceed as directed under 12, and calculate results as  $\text{As}_2\text{O}_3$ .

**55 TOTAL ZINC OXIDE—OFFICIAL**

Transfer a 25 cc aliquot of the soln prepared for the determination of total  $\text{As}_2\text{O}_3$ , 52, to a beaker and add 5 cc of HCl. If there is much Fe present, reduce it by adding a little  $\text{NaHSO}_3$  and heating on a steam bath until the odor of  $\text{SO}_2$  has practically disappeared. Cool, dilute to about 100 cc, and proceed as directed under 17, beginning with "add 35–40 cc of the Hg-thiocyanate reagent with vigorous stirring."

**COPPER CARBONATE****COPPER OXIDE****56 Electrolytic Method—Official**

Weigh 0.5 g of the sample, transfer to a 150 cc Pt dish or 150 cc beaker, and dissolve in 25 cc of  $\text{HNO}_3$  (1+4). Dilute to about 100 cc and determine the Cu by electrolysis, as directed under 14, beginning with "electrolyze, using a rotating anode and a current of about 3 amperes."

**57 Thiosulfate Volumetric Method—Official**

Dissolve 0.25–0.5 g of the sample in 25 cc of  $\text{HNO}_3$  (1+4), dilute to about 50 cc, and proceed as directed under 15, beginning with "add  $\text{NH}_4\text{OH}$  in excess."

## BORDEAUX MIXTURE

58

## MOISTURE—OFFICIAL

(a) *Powder*.—Dry 2 g to constant weight at 105–110° and report the loss as moisture.

(b) *Paste*.—Heat about 100 g in an oven at 90–100° until dry enough to powder readily and note the loss in weight. Powder this partially dried sample and determine the remaining moisture in 2 g, as directed under (a). Determine CO<sub>2</sub>, as directed under 60, both in the original paste and in this partially dried sample. Calculate the total moisture by the following formula:

$$M = a + \frac{(100 - a)(b + c)}{100} - d, \text{ in which}$$

$M$  = percentage of total moisture in original paste;

$a$  = percentage of loss in weight of original paste during first drying;

$b$  = percentage of loss in weight of partially dried paste during second drying;

$c$  = percentage of CO<sub>2</sub> remaining in partially dried paste after first drying; and

$d$  = percentage of total CO<sub>2</sub> in original paste.

CARBON DIOXIDE<sup>18</sup> OFFICIAL

59

## APPARATUS

Use a 200 cc Erlenmeyer flask closed with a 2-holed stopper; in one hole fit a dropping funnel, allowing the stem to extend almost to the bottom of the flask, and thru the other hole pass the outlet of a condenser, which is inclined upward at an angle of 30° from the horizontal. Connect the upper end of the condenser with a CaCl<sub>2</sub> tube, which in turn is connected with a double U-tube filled in the middle with pumice fragments, previously saturated with CuSO<sub>4</sub> soln (20% CuSO<sub>4</sub> · 5H<sub>2</sub>O) and subsequently dehydrated, and with CaCl<sub>2</sub> at either end. Then connect two weighed U-tubes for absorbing the CO<sub>2</sub>, the first filled with porous soda-lime, and the second,  $\frac{1}{3}$  with soda-lime and  $\frac{2}{3}$  with CaCl<sub>2</sub>, placing the CaCl<sub>2</sub> at the exit end of the train. Attach a Geissler bulb, partly filled with H<sub>2</sub>SO<sub>4</sub>, to the last U-tube to show the rate of gas flow, and connect an aspirator with the Geissler bulb to draw air thru the apparatus. Connect an absorption tower filled with soda-lime to the mouth of the dropping funnel to remove CO<sub>2</sub> from the air entering the apparatus.

60

## DETERMINATION

Weigh 2 g of the powder or 10 g of the paste into the Erlenmeyer flask and add about 20 cc of H<sub>2</sub>O. Attach the flask to the apparatus, omitting the 2 weighed U-tubes, and draw CO<sub>2</sub>-free air thru the apparatus until it displaces the original air. Then attach the weighed U-tubes as directed under 59; close the stopcock of the dropping funnel; pour into it 50 cc of HCl (1+4); reconnect with the soda-lime tower; and allow the acid to flow into the Erlenmeyer flask, slowly if there is much CO<sub>2</sub>, rapidly if there is little. When effervescence diminishes, place a low Bunsen flame under the flask and start a flow of H<sub>2</sub>O thru the condenser, allowing a slow current of air to flow thru the apparatus at the same time. Maintain a steady but quiet ebullition and a slow air current thru the apparatus. Boil for a few min. after the H<sub>2</sub>O has begun to condense in the condenser, then remove the flame and continue the aspiration of air at the rate of about 2 bubbles per second until the apparatus is cool. Disconnect the weighed absorption tubes, cool in the balance case, and weigh. The increase in weight is CO<sub>2</sub>.

## COPPER

61 *Electrolytic Method—Official*

Dissolve 2 g of the powdered sample in 25 cc of  $\text{HNO}_3$  (1+4), dilute to 100 cc, and electrolyze, using a rotating spiral anode and a current of about 3 amperes, as directed under 14, beginning with "Wash into a weighed 150 cc Pt dish."

62 *Thiosulfate Volumetric Method—Official*

Dissolve 2 g of the powdered sample in about 25 cc of  $\text{HNO}_3$  (1+4), dilute to 50 cc, add  $\text{NH}_4\text{OH}$  in excess, and heat; then, without removing the precipitate that has formed, boil off the excess of  $\text{NH}_3$ , add 3–4 cc of acetic acid, cool, add 10 cc of 30% KI soln, and titrate as directed under 15, beginning with "titrate with standard thiosulfate soln."

## BORDEAUX MIXTURE WITH PARIS GREEN

## 63 MOISTURE—OFFICIAL

Proceed as directed under 58.

## 64 CARBON DIOXIDE—OFFICIAL

Proceed as directed under 60.

## 65 TOTAL ARSENIC—OFFICIAL

Proceed as directed under 5 or 8, using 2 g of the sample and calculating the results as  $\text{As}_2\text{O}_3$ .

## 66 TOTAL ARSENIOS OXIDE—OFFICIAL

Proceed as directed under 23, using 0.5–1.0 g of the sample.

## 67 WATER-SOLUBLE ARSENIOS OXIDE—OFFICIAL

Proceed as directed under 27, using 2 g of the sample and slightly acidifying the aliquot used with  $\text{HCl}$  (1+4) before adding the excess of  $\text{NaHCO}_3$ .

## COPPER

68 *Electrolytic Method I—Official*

Proceed as directed under 14.

69 *Electrolytic Method II—Official*

(Preferred Method.)

Dissolve 2 g of the powdered sample in a 150 cc beaker with 5 cc of  $\text{HNO}_3$ , add 25 cc of a 3% soln of  $\text{H}_2\text{O}_2$ , and warm on a steam bath for 5–10 min. Add 25 cc more of  $\text{H}_2\text{O}_2$  soln; dilute to 100 cc; and electrolyze, using a weighed gauze cathode, a rotating paddle anode, and a current of 2–3 amperes. At the end of about 20 min., add 15–20 cc more of the  $\text{H}_2\text{O}_2$  soln. After all Cu is deposited, which should not require more than 45 min., and while the current is still flowing, wash the deposit with  $\text{H}_2\text{O}$  by siphoning. Then interrupt the current, rinse with alcohol, dry for a few min. in an oven, weigh, and calculate the percentage of Cu. (Do not pass the current for more than 5–10 min. after all Cu is deposited without adding more of the  $\text{H}_2\text{O}_2$  soln.)

70 *Thiosulfate Volumetric Method—Official*

Proceed as directed under 15.

## BORDEAUX MIXTURE WITH LEAD ARSENATE

## 71 MOISTURE—OFFICIAL

Proceed as directed under 58.

## 72 CARBON DIOXIDE—OFFICIAL

Proceed as directed under 60.

## 73 TOTAL ARSENIC—OFFICIAL

Proceed as directed under 5 or 8, using 2 g of the sample and calculating the results as  $\text{As}_2\text{O}_5$ .

## 74 WATER-SOLUBLE ARSENIC—OFFICIAL

Proceed as directed under 12 and calculate the results as  $\text{As}_2\text{O}_5$ .

## COPPER

75 *Electrolytic Method—Official*

Proceed as directed under 14.

76 *Thiosulfate Volumetric Method—Official*

Proceed as directed under 15.

## 77 LEAD OXIDE—OFFICIAL

Proceed as directed under 13.

## LEAD OXIDE AND COPPER

*Electrolytic Method<sup>1a</sup>—Official*

## 78 APPARATUS

*Electrodes.*—Cathode, a cylindrical platinum electrode, either gauze or plate, approximately 50 mm high and 25 mm in diameter. Anode, gauze or plate, approximately 50 mm high and 50 mm in diameter. This electrode should be sandblasted.

## 79 DETERMINATION

Weigh 1 g of the powdered sample and transfer to a 250 cc beaker. Add 15 cc of HCl and 5 cc of HBr, and evaporate to dryness on a steam bath. Repeat the treatment, and finally, to remove the last traces of As, add 20 cc of the HCl and again evaporate to dryness.

To the residue add 25 cc of  $\text{H}_2\text{O}$  and 15 cc of  $\text{HNO}_3$  and heat to boiling. Cautiously boil until most of the bromides and some of the chlorides are expelled (characterized by changes in color, first from brown to green, and then to blue). Evaporate to dryness on a steam bath. Add 10 cc of  $\text{H}_2\text{O}$  and 15 cc of  $\text{HNO}_3$ , and again evaporate to dryness. Take up in 50 cc of  $\text{H}_2\text{O}$  and 12 cc of  $\text{HNO}_3$ , and heat until all salts are in solution. (It is not necessary to filter off any siliceous material that may be present.) Dilute to 200 cc and electrolyze overnight, using a current of 0.15 ampere and a potential of 1.5–2 volts.

Add 15–20 cc of  $\text{H}_2\text{O}$  to the electrolyte and continue to use the current for a few minutes. If there is no further deposition on the newly exposed surfaces of the electrodes, wash them several times with  $\text{H}_2\text{O}$  without breaking the current. Finally break the current and wash once with methyl or ethyl alcohol. Dry the electrodes

in an oven at 105–110° for 1 hour. The increase in weight of the cathode represents the Cu present in the sample, and the increase in weight of the anode represents the lead as PbO<sub>2</sub>. From the increased weight of the cathode, calculate the percentage of Cu in the sample. As the PbO<sub>2</sub> is not completely anhydrous, multiply the weight found by the factor 0.9267, and calculate the percentage of PbO in the sample.

#### BORDEAUX MIXTURE WITH CALCIUM ARSENATE

##### 80 MOISTURE—OFFICIAL

Proceed as directed under 58.

##### 81 CARBON DIOXIDE—OFFICIAL

Proceed as directed under 60.

##### 82 TOTAL ARSENIC—OFFICIAL

Proceed as directed under 5 or 8, using 2 g of the sample and calculating the results as As<sub>2</sub>O<sub>3</sub>.

##### 83 WATER-SOLUBLE ARSENIC OFFICIAL

Proceed as directed under 12 and calculate the results as As<sub>2</sub>O<sub>3</sub>.

#### COPPER

##### 84 *Electrolytic Method I—Official*

Proceed as directed under 14.

##### 85 *Electrolytic Method II—Official*

Proceed as directed under 69.

##### 86 *Thiosulfate Volumetric Method—Official*

Proceed as directed under 15.

#### SODIUM AND POTASSIUM CYANIDES

##### CYANOGEN<sup>20</sup> OFFICIAL

##### 87 REAGENT

*Silver nitrate soln.*—0.1 N. Standardize against pure NaCl by titration, using chromate indicator; or gravimetrically, weighing the chloride.

##### 88 DETERMINATION

Break the sample into small lumps in a mortar (do not grind). Weigh quickly about 5 g in a weighing bottle and wash into a 500 cc volumetric flask containing about 200 cc of H<sub>2</sub>O. Add a little PbCO<sub>3</sub> to precipitate any sulfides that may be present, dilute to the mark with H<sub>2</sub>O, mix thoroly, and filter thru a dry filter. Transfer a 50 cc aliquot to a 400 cc beaker; add 200 cc of H<sub>2</sub>O, 5 cc of NaOH soln (100 g to 1 liter of H<sub>2</sub>O) and 10 drops of saturated KI soln (or a few crystals); and titrate to a faint opalescence with the AgNO<sub>3</sub> soln. (In making this titration, it is advantageous to have the beaker over a black surface.) From the number of cc of 0.1 N AgNO<sub>3</sub> soln used calculate the percentage of CN in the sample. The reaction is represented by the equation: 2NaCN + AgNO<sub>3</sub> = NaCN·AgCN + NaNO<sub>3</sub>; hence 1 cc of 0.1 N AgNO<sub>3</sub> soln = 0.005202 g of CN.

CHLORINE<sup>21</sup>*Method I—Official*

89

## REAGENTS

(a) *Ammonium or potassium thiocyanate soln.*—0.1 N. Adjust by titrating against the 0.1 N AgNO<sub>3</sub> soln, 87.

(b) *Ferric indicator.*—A saturated soln of ferric ammonium alum.

90

## DETERMINATION

Transfer a 50 cc aliquot of the prepared soln, 88, to a beaker, dilute with an equal volume of H<sub>2</sub>O, add 1–2 cc of 40% chloride-free HCHO soln, stir well, and let stand for 15 min. Acidify with HNO<sub>3</sub> (1+1) (5 cc), add a measured volume of 0.1 N AgNO<sub>3</sub> soln, 87, sufficient to give an excess, stir well, filter, wash and titrate the excess of Ag in the combined filtrate and washings with the 0.1 N thiocyanate soln, using the ferric indicator. From the number of cc of 0.1 N AgNO<sub>3</sub> soln, less the number of cc of thiocyanate soln used, calculate the percentage of Cl in the sample.

91

*Method II<sup>21</sup>—Official*

Transfer a 50 cc aliquot of the prepared soln, 88, to a distilling flask, dilute to 100–150 cc, acidify with a slight excess of acetic acid, and distil, passing the vapors thru a condenser, the delivery end of which dips into a soln of NaOH, to absorb the HCN. After all the HCN has been driven off (50 cc of distillate), wash the liquid remaining in the distilling flask into a beaker, add 5 cc of HNO<sub>3</sub> (1+1) and then a measured volume of 0.1 N AgNO<sub>3</sub> soln, 87, sufficient to give an excess, stir well, filter, wash, and titrate the excess of Ag in the combined filtrate and washings with thiocyanate soln, 89(a), using ferric indicator, 89(b). From the number of cc of 0.1 N AgNO<sub>3</sub> soln, less the number of cc of 0.1 N thiocyanate soln used, calculate the percentage of Cl in the sample.

## CALCIUM CYANIDE

CYANOGEN<sup>22</sup>—OFFICIAL

92

## REAGENT

*Soda-lead.*—Dissolve 20 g of Pb acetate in H<sub>2</sub>O, dilute to 1 liter, and add 200 g of chloride-free Na<sub>2</sub>CO<sub>3</sub>.

93

## DETERMINATION

Place about 200 cc of H<sub>2</sub>O in a 500 cc volumetric flask and carefully dry the neck of the flask. Weigh about 5 g of the sample in a weighing bottle and transfer to the flask with the least possible exposure to the air. Wash the mixture down into the flask and mix by whirling until soln is complete and the small quantity of CaC<sub>2</sub> has been decomposed. Then add 25 cc of the soda-lead, or a quantity sufficient to remove sulfides; close the flask with a rubber stopper and shake thoroly, preferably for 30 min. Dilute to the mark, mix, and filter thru a dry filter. Transfer a 50 cc aliquot to a 400 cc beaker and proceed as directed under 88, beginning with "Add 200 cc of H<sub>2</sub>O." 1 cc of 0.1 N AgNO<sub>3</sub> soln = 0.005202 g of CN. To obtain the percentage of Ca(CN)<sub>2</sub>, multiply the percentage of CN by the factor 1.7703.

CHLORINE<sup>22</sup>

94

*Method I—Official*

Transfer a 50 cc aliquot of the prepared soln, 93, to a beaker, and proceed as directed under 90.

95

*Method II<sup>22</sup>—Official*

Transfer a 50 cc aliquot of the prepared soln, 93, to a distilling flask, and proceed as directed under 91.

**SOAP****MOISTURE<sup>23</sup>**

96

*Xylene Distillation Method—Official*

Weigh about 20 g of the sample into a 300–500 cc flask; add 50 cc of xylene (technical grade is satisfactory); and, to prevent foaming, add about 10 g of lump

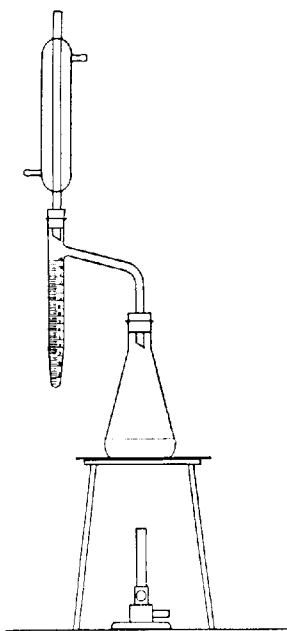


FIG. 7.—DEAN AND STARK DISTILLING TUBE RECEIVER

rosin (do not use powdered). Distil into a Dean and Stark type distilling tube receiver<sup>24</sup> and continue the distillation until no more  $H_2O$  collects in the receiver. Allow the contents of the tube to cool to room temp., read the volume of  $H_2O$  under the xylene in the tube, and from this volume calculate the percentage of  $H_2O$  in the sample.

97

**POTASSIUM AND SODIUM<sup>25</sup>—OFFICIAL**

Dissolve about 5 g of the soap in  $H_2O$ , decompose with  $HCl$  (1+4), filter off the  $H_2O$ , and wash the fat with cold  $H_2O$ . Determine both K and Na in the filtrate as directed under XII, 14 and 15.

## MINERAL OILS

98

UNSULFONATED RESIDUE<sup>26</sup>—OFFICIAL

Pipet 5 cc of the oil into a Babcock cream bottle about 15 cm (6 inches) long (either the 9 g 50% or the 18 g 30% type). To reduce the viscosity of heavy oils, warm the pipet after a preliminary draining by drawing it several times thru the flame of a Bunsen burner and drain thoroly. If greater accuracy is desired, weigh the measured charge and calculate its exact volume from the weight and sp. gr. of the oil. Add slowly 20 cc of 38 N H<sub>2</sub>SO<sub>4</sub>, VIII, 18, gently shaking or rotating the bottle and taking care that the temp. does not rise above 60°. Cool in ice H<sub>2</sub>O if necessary. When the mixture no longer develops heat on shaking, agitate thoroly, place the bottle in a water bath, and heat at 60–65° for 10 min., keeping the contents of the bottle thoroly mixed by shaking vigorously for a period of 20 seconds at 2 min. intervals. Remove the bottle from the bath and fill with H<sub>2</sub>SO<sub>4</sub> until the oil rises into the graduated neck. Centrifuge for 5 min. (or longer if necessary to obtain a constant volume of oil) at 1200–1500 r.p.m. Read the volume of unsulfonated residue from the graduations on the neck of the bottle and, to convert to cc, multiply the reading from the 9 g 50% bottle by 0.1 and that from the 18 g 30% bottle by 0.2. From the result thus obtained calculate the percentage by volume of the unsulfonated oil.

## MINERAL OIL—SOAP EMULSIONS

WATER<sup>27</sup>

99

*Xylene Distillation Method—Official*

Weigh about 25 g of the sample and proceed as directed in 96, except to use a smaller quantity of rosin.

100

TOTAL OIL<sup>28</sup>—OFFICIAL

Weigh about 10 g of the sample into a Babcock cream bottle, 98. Dilute with about 10 cc of hot H<sub>2</sub>O and add 5–10 cc of H<sub>2</sub>SO<sub>4</sub> (1+1). Set the bottle in a hot water bath for about 5 min. to hasten the separation of the oil, add sufficient saturated NaCl soln to bring the oil layer within the graduations on the neck of the bottle, whirl at a rate of 1200 r.p.m. for 5 min., and allow to cool. Read the volume of the oil layer, determine its density, and from these values calculate its weight and percentage. From this percentage value, deduct the percentage of fatty acids (and phenols if present) determined separately, to obtain the percentage of oil in the sample.

101

SOAP<sup>27</sup>—OFFICIAL

(In this method error will result if the apparent molar weight of the fatty acids varies appreciably from that of oleic acid.)

Weigh 20 g of the sample into a separatory funnel, add 60 cc of petroleum ether, and extract the mixture once with 20 cc and four times with 10 cc of 50% alcohol. Break the emulsion if necessary with 1 or 2 cc of a 20% soln of NaOH, allowing the soln to run down the side of the separatory funnel, which is then gently twirled and allowed to stand for a few min. Draw off the alcoholic layers and wash them successively thru petroleum ether contained in 2 other separatory funnels. Combine the alcoholic extracts in a beaker and evaporate on a steam bath to remove the alcohol. Dissolve the residue in about 100 cc of H<sub>2</sub>O made alkaline with NaOH. Transfer to a separatory funnel, acidify with HCl or H<sub>2</sub>SO<sub>4</sub>, extract 3 times with ether, and wash



the ether extracts twice with  $H_2O$ . Combine the ether extracts, evaporate in a weighed beaker on a steam bath, and weigh as fatty acids. From the weight of fatty acids calculate the percentage of soap in the sample as Na- or K-oleate.

102

**UNSULFONATED RESIDUE—OFFICIAL**

Using 5 cc of the recovered oil, proceed as directed under 98.

103

**ASH<sup>29</sup>—OFFICIAL**

Evaporate 10 g of the sample, or more if necessary, in a Pt dish; ignite, and leach the charred mass with  $H_2O$ . Ignite the residue, add the leachings, evaporate to dryness, ignite, and weigh. From this weight calculate the percentage of ash. Test the ash for Cu, Ca,  $CaF_2$ , etc.

**SODA LYE****CARBONATE AND HYDROXIDE<sup>30</sup>—OFFICIAL**

104

**REAGENTS**

(a) *Phenolphthalein indicator*.—Dissolve 1 g of phenolphthalein in 100 cc of neutralized alcohol 95% by volume.

(b) *Barium chloride soln*.—Dissolve 100 g of  $BaCl_2 \cdot 2H_2O$  and dilute to 1 liter.

105

**DETERMINATION**

Weigh about 10 g of the sample from a weighing bottle, dissolve in  $CO_2$ -free  $H_2O$ , and dilute to a definite volume. Titrate an aliquot of this soln with the 0.5 *N* HCl, II, 19 (a), using the methyl orange indicator, 3 (f), and note the total alkalinity thus found. Transfer an equal aliquot to a volumetric flask and add enough of the  $BaCl_2$  soln to precipitate all the carbonate, avoiding any unnecessary excess. Dilute to the mark with  $CO_2$ -free  $H_2O$ , stopper, shake, and set aside. When the liquid becomes clear, pipet off one-half and titrate with the 0.5 *N* HCl, using the phenolphthalein indicator. The number of cc of 0.5 *N* acid required for this titration, multiplied by 2, gives the number of cc of 0.5 *N* acid equivalent to the NaOH present in the original aliquot. The difference between this figure and the number of cc of 0.5 *N* HCl required for the total alkalinity represents the number of cc of 0.5 *N* acid equivalent to the  $Na_2CO_3$  present in the aliquot. Calculate the percentages of  $Na_2CO_3$  and NaOH present in the sample.

**TOBACCO AND TOBACCO EXTRACT****NICOTINE***Silicotungstic Acid Method<sup>31</sup>—Official*

106

**REAGENTS**

(a) *Silicotungstic acid soln*.—Dissolve 120 g of silicotungstic acid ( $4H_2O \cdot SiO_2 \cdot 12WO_3 \cdot 22H_2O$ ) in  $H_2O$  and dilute to 1 liter. (Of the several silicotungstic acids,  $4H_2O \cdot SiO_2 \cdot 10WO_3 \cdot 3H_2O$  and  $4H_2O \cdot SiO_2 \cdot 12WO_3 \cdot 20H_2O$  do not give crystalline precipitates with nicotine and should not be used.)

107

**DETERMINATION**

Weigh such a quantity of the preparations as will contain preferably 0.1–1.0 g of nicotine (if the sample contains very little nicotine, i.e., about 0.1%, do not increase the quantity to the point where it interferes with the distillation); wash

with  $\text{H}_2\text{O}$  into a 500 cc round-bottomed distillation flask; and add a little paraffin to prevent frothing, a few small pieces of pumice, and a slight excess of  $\text{NaOH}$  soln, 3(d), using phenolphthalein indicator, 104(a). Distil rapidly in a current of steam thru a well-cooled condenser, connected by means of an adapter with a suitable flask containing 10 cc of  $\text{HCl}$  (1+4). When distillation is well under way, heat the distillation flask to reduce the volume of the liquid as far as practicable without bumping or undue separation of insoluble matter. Distil until a few cc of the distillate shows no cloud or opalescence when treated with a drop of the silicotungstic acid and a drop of  $\text{HCl}$  (1+4). Confirm the alkalinity of the residue in the distillation flask with the phenolphthalein indicator. Make the distillate, which may amount to 1000–1500 cc, to a convenient volume (the soln may be concentrated on the steam bath without loss of nicotine); mix well; and pass thru a large dry filter if not clear. Test a portion with methyl orange, 3(f), to confirm its acidity. Pipet an aliquot, containing about 0.1 g of nicotine, into a beaker (if the samples contain very small quantities of nicotine, an aliquot containing as little as 0.01 g of nicotine may be used); add to each 100 cc of liquid 3 cc of the dilute  $\text{HCl}$ , or more if the necessity is indicated by the test with methyl orange, and 1 cc of the silicotungstic acid for each 0.01 g of nicotine supposed to be present. Stir thoroly and let stand overnight. Before filtering, stir the precipitate to see that it settles quickly and is in crystalline form, filter on an ashless filter, and wash with cold  $\text{HCl}$  (1+1000). Transfer the paper and precipitate to a weighed Pt crucible, dry carefully, and ignite until all C is destroyed. Finally heat over a Meker burner for not more than 10 min. Weight of residue  $\times 0.1140$  = weight of nicotine present in the aliquot.

## FORMALDEHYDE SOLUTIONS

### FORMALDEHYDE

#### *Hydrogen Peroxide Method*<sup>52</sup>. Official

#### 108

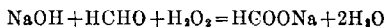
##### REAGENTS

- (a) *Sulfuric acid*.—1 N. Prepare as directed under II, 19(b).
- (b) *Sodium hydroxide soln*.—1 N. Standardize against (a), using the litmus or bromothymol blue indicator. 1 cc = 30.02 mg. of  $\text{HCHO}$ .
- (c) *Hydrogen peroxide soln*.—Commercial, containing about 3% of  $\text{H}_2\text{O}_2$ . If acid, neutralize with the 1 N  $\text{NaOH}$ , (b), using litmus or bromothymol blue indicator.
- (d) *Litmus indicator*.—A soln of purified litmus of sufficient strength that 3 drops will impart a distinct blue color to 50 cc of  $\text{H}_2\text{O}$ .
- (e) *Bromothymol blue indicator*.—Dissolve 1 g of bromothymol blue in 500 cc of alcohol, 50% by volume.

#### 109

##### DETERMINATION

Measure 50 cc of the 1 N  $\text{NaOH}$  into a 500 cc Erlenmeyer flask and add 50 cc of the  $\text{H}_2\text{O}_2$ . Then add a weighed quantity, about 3 g, of the sample, allowing the point of the weighing pipet to reach nearly to the liquid in the flask. Place a funnel in the neck of the flask and heat on a steam bath for 5 min., shaking occasionally. Remove from the steam bath, wash the funnel with  $\text{H}_2\text{O}$ , cool the flask to about room temp., and titrate the excess  $\text{NaOH}$  with the 1 N acid, using the bromothymol blue or litmus indicator. (It is necessary to cool the flask before titration with the acid in order to obtain a sharp end point with the litmus.) From the amount of 1 N  $\text{NaOH}$  consumed and the weight of the sample calculate the percentage of  $\text{HCHO}$ , according to the following equation:



If the HCHO soln contains an appreciable quantity of free acid, titrate a separate portion and calculate the acidity as percentage of formic acid. Make correction for this acidity in calculating the percentage of HCHO.

*Cyanide Method*<sup>22</sup>—Official

(Applicable only to dilute solutions.)

110

## REAGENT

*Potassium cyanide soln.*—Dissolve 3.1 g of KCN in 500 cc of H<sub>2</sub>O.

111

## DETERMINATION

Treat 15 cc of 0.1 *N* AgNO<sub>3</sub> soln, 87, with 6 drops of HNO<sub>3</sub> (1+1) in a 50 cc volumetric flask, add 10 cc of the KCN soln, dilute to the mark, shake well, filter thru a dry filter, and titrate 25 cc of the filtrate with 0.1 *N* NH<sub>4</sub>SCN, 89(a), as directed under XII, 37. Acidify another 15 cc portion of 0.1 *N* AgNO<sub>3</sub> with 6 drops of the dilute HNO<sub>3</sub> and treat with 10 cc of the KCN soln to which has been added a measured quantity (the weight must be calculated from the sp. gr.) of the sample containing not over 25 mg of HCHO. Dilute to 50 cc, filter, and titrate a 25 cc aliquot with the 0.1 *N* NH<sub>4</sub>SCN for the excess of Ag as before. The difference between the cc of NH<sub>4</sub>SCN used in these 2 titrations × 2 = the cc of 0.1 *N* NH<sub>4</sub>SCN corresponding to the KCN used by the HCHO. Calculate the percentage of HCHO present. 1 cc of 0.1 *N* NH<sub>4</sub>SCN = exactly 3 mg of HCHO.

## LIME-SULFUR SOLUTIONS

TOTAL SULFUR<sup>23</sup>—OFFICIAL

112

## PREPARATION OF SAMPLE

Weigh about 10 g of the soln, transfer to a 250 cc volumetric flask, and immediately dilute to the mark with recently boiled and cooled H<sub>2</sub>O. Mix thoroly and transfer to a number of small bottles, filling them completely and avoiding contact of the soln with air as much as possible. Stopper the bottles, seal with paraffin, and preserve in a dark, cool place.

113

## DETERMINATION

(Sulfur-free reagents should be used.)

Dissolve 2-3 g of Na<sub>2</sub>O<sub>2</sub> in 50 cc of cold H<sub>2</sub>O in a 250 cc beaker. Transfer a 10 cc aliquot of the prepared soln to this aqueous soln of Na<sub>2</sub>O<sub>2</sub>, keeping the tip of the pipet constantly just under the surface of the liquid until necessary to raise it for drainage at the end. Use a clean dry pipet for measuring each portion. Cover the beaker with a watch-glass and heat on a steam bath, with occasional stirring, until all the S is oxidized to sulfate (indicated by the disappearance of the yellow color). Wash off the watch-glass and the sides of the beaker, acidify with HCl (1+4), evaporate to complete dryness, treat with H<sub>2</sub>O acidified with HCl, boil, and filter to remove SiO<sub>2</sub>. Dilute the filtrate to 300 cc, add 50 cc of HCl, heat to boiling, and add 10% BaCl<sub>2</sub> soln (11 cc for 1 g of BaSO<sub>4</sub>) with constant stirring, at such a rate that about 4 min. is required for running in the necessary quantity. (The rate may be regulated by attaching a suitable capillary tip to a buret containing the BaCl<sub>2</sub> soln.) Evaporate to dryness on a steam bath, take up with hot H<sub>2</sub>O, filter thru a

quantitative filter, wash until free from chlorides, ignite carefully, and heat to constant weight over a Bunsen burner. Calculate the percentage of S from the weight of  $\text{BaSO}_4$ , using the factor 0.1374.

#### MONOSULFIDE EQUIVALENT<sup>38</sup>—TENTATIVE

114

##### REAGENT

*Iodine soln.*—0.1 N. Prepare as directed under 3(b), using 12.7 g of I and 25 g of KI.

115

##### DETERMINATION

Dilute 10 cc of the prepared soln, 112, to about 30 cc with recently boiled and cooled  $\text{H}_2\text{O}$  and titrate with the 0.1 N I soln until the yellow color just disappears. (There should be no difficulty in determining this end point; if there is, a small crystal of Na nitroprusside may be used, but it must not be added until the end point is practically reached, because the blue color, if well developed, cannot be destroyed except by an excess of I.) From the number of cc of 0.1 N I soln used calculate the percentage of monosulfide equivalent. 1 cc of 0.1 N I = 0.0016 g of S as monosulfide equivalent.

#### THIOSULFATE SULFUR

##### *Zinc Chloride Method*<sup>34</sup>—Official

116

##### REAGENT

*Ammoniacal zinc chloride soln.*—Dissolve 50 g of pure  $\text{ZnCl}_2$  in about 500 cc of  $\text{H}_2\text{O}$ , add 125 cc of  $\text{NH}_4\text{OH}$  and 50 g of  $\text{NH}_4\text{Cl}$ , and dilute to 1 liter.

117

##### DETERMINATION

To 50 cc of  $\text{H}_2\text{O}$  in a 200 cc volumetric flask, add, in the manner indicated under 113, 50 cc of the soln prepared as directed under 112. Add a slight excess of the ammoniacal  $\text{ZnCl}_2$  soln and dilute to the mark. Shake thoroly and filter thru a dry filter. To 100 cc of the filtrate add a few drops of methyl orange or methyl red indicator, 3(f) or II, 19(i), and exactly neutralize with 0.1 N HCl. Titrate the neutral soln with 0.05 N I soln, 3(b), using a few drops of starch indicator, 3(e). From the number of cc of I soln used calculate the percentage of thiosulfate S present. As the value of the I soln is given in terms of  $\text{As}_2\text{O}_3$ , multiply this value by 1.296 to obtain the equivalent of thiosulfate S.

118

##### *Iodine Titration Method*<sup>36</sup>—Tentative

Continue the titration of the soln used in the determination of the monosulfide equivalent, 115, with the 0.1 N I soln, letting the I act as its own indicator until a small drop produces a slight permanent coloration. From the number of cc of 0.1 N I used calculate the percentage of thiosulfate S. 1 cc of 0.1 N I = 0.0064 g of S as thiosulfate.

#### SULFIDE SULFUR

119

##### *Zinc Chloride Method*<sup>34</sup>—Official

To 10–15 cc of  $\text{H}_2\text{O}$  in a small beaker add, in the manner indicated under 113, a 10 cc aliquot of the soln prepared as directed under 112. Calculate the quantity of ammoniacal  $\text{ZnCl}_2$  soln, 116, necessary to precipitate all the S in the aliquot and add a slight excess. Stir thoroly, filter, wash the precipitate twice with cold  $\text{H}_2\text{O}$ ,

and transfer the filter paper and precipitate to the beaker in which the precipitation was made. Cover with  $\text{H}_2\text{O}$ , disintegrate the paper with a glass rod, and add about 3 g of  $\text{Na}_2\text{O}_2$ , keeping the beaker well covered with a watch-glass. Warm on a steam bath with frequent shaking until all the S is oxidized to sulfate, adding more  $\text{Na}_2\text{O}_2$  if necessary. Make slightly acid with  $\text{HCl}$  (1 + 4), filter to remove shreds of the filter paper, wash thoroly with hot  $\text{H}_2\text{O}$ , and determine the S in the filtrate as directed under 113.

120

*Iodine Titration Method<sup>35</sup>—Tentative*

Allow the soln from 118 to stand several hours with occasional stirring, or acidify with a few drops of  $\text{HCl}$  (1 + 4); warm gently with stirring, filter, and wash thoroly with warm  $\text{H}_2\text{O}$ . Place the filter paper with the S in a small vessel and dissolve the S in about 15 cc of  $\text{NaOH}$  soln, 3(d), by heating gently on a steam or water bath for 1–1.5 hours (do not boil). Keep the flask covered and shake gently a few times during the digestion to remove the S from the sides. Oxidize by adding 2–3 g of  $\text{Na}_2\text{O}_2$  dissolved in 50 cc of cold  $\text{H}_2\text{O}$  and complete the determination as directed under 113, beginning with "Cover the beaker with a watch-glass."

121

*Indirect Method—Tentative*

The difference between the total S and the sum of the thiosulfate S and sulfate S is the sulfide S.

**SULFATE SULFUR**

122

*Zinc Chloride Method—Official*

Slightly acidify the soln from the determination of thiosulfate S, 117, with  $\text{HCl}$  (1 + 4), heat to boiling, add slowly and with constant stirring a slight excess of a 10%  $\text{BaCl}_2$  soln, boil for 30 min., allow to stand overnight, and filter. Calculate the S from the weight of  $\text{BaSO}_4$ , and report as % of sulfate S.

123

*Iodine Titration Method<sup>35</sup>—Tentative*

To the filtrate from the determination of thiosulfate S, 118, add several drops of  $\text{HCl}$ , precipitate in the cold with 5 cc of 10%  $\text{BaCl}_2$  soln, allow to stand overnight, and filter. Calculate the S from the weight of  $\text{BaSO}_4$ , and report as percentage of sulfate S.

124

**TOTAL LIME<sup>31</sup>—OFFICIAL**

To 25 cc of the soln, prepared as directed under 112, add 10 cc of  $\text{HCl}$ , evaporate to dryness on a steam bath, treat with  $\text{H}_2\text{O}$  and a few cc of  $\text{HCl}$  (1 + 4), warm until all the  $\text{CaCl}_2$  is dissolved, and filter to remove S and any  $\text{SiO}_2$  that may be present. Dilute the filtrate to a volume of 200–250 cc, heat to boiling, add a few cc of  $\text{NH}_4\text{OH}$  in excess, and then an excess of a saturated soln of  $(\text{NH}_4)_2\text{C}_2\text{O}_4$ . Continue the boiling until the precipitated  $\text{CaC}_2\text{O}_4$  assumes a well defined granular form, allow to stand for an hour, filter, and wash a few times with hot  $\text{H}_2\text{O}$ . Ignite in a Pt crucible over a blast lamp to constant weight and calculate to percentage of  $\text{CaO}$ .

**ORGANIC MERCURIAL SEED DISINFECTANTS****MERCURY***Volatilization Method<sup>36</sup>—Official*

125

**APPARATUS**

The apparatus (Fig. 8) consists of 2 flanged crucibles that can be clamped mouth to mouth by means of 2 rings and screws. The lower crucible is made of Fe and the

upper one of Au. The opening of the Au crucible is slightly larger than that of the other, so that there will be no tendency for the Hg to lodge in the joint between the two flanges. The Au crucible is fitted with a cooling device by which  $H_2O$  may be slowly circulated through a large tube attached to it by Gooch tubing. The assembled apparatus rests on an asbestos board having a hole just large enough to receive the crucible.

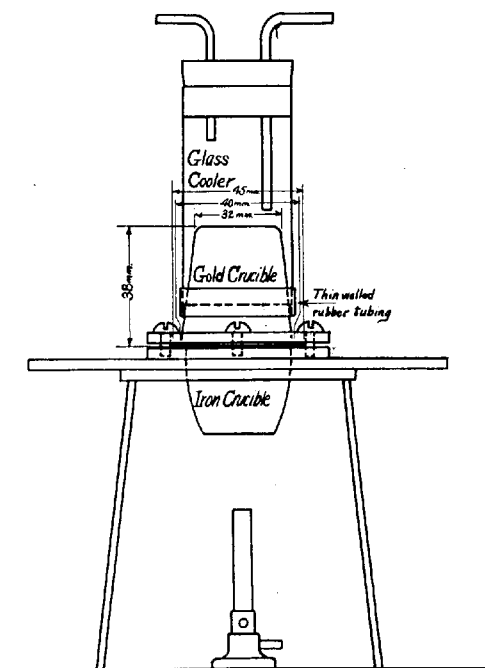


FIG. 8.—APPARATUS TO BE USED IN VOLATILIZATION METHOD

126

#### DETERMINATION

Weigh 1 g of the sample into the Fe crucible and mix it thoroly with 5 g of anhydrous  $Na_2CO_3$ . Cover the mixture with a thin layer of  $Na_2CO_3$  and then with 10 g of finely powdered  $BaCO_3$ . Put the weighed Au crucible in place, clamp the two together, set the Fe crucible in place in the asbestos board, start the cooling  $H_2O$ , and gently heat the Fe crucible. Do not run the  $H_2O$  too fast because the Hg amalgamates best with the Au crucible if the temp. is allowed to rise to about  $50^\circ$ . Heat below red heat for 30 min., cool, remove the Au crucible, wash it with 95% alcohol, dry with the heat of the hand, and then place in a  $CaCl_2$  desiccator until it attains constant weight. Calculate the increase in weight of the Au crucible as percentage of metallic Hg in the sample. If the product contains more than 12% Hg, use less than 1 g because the Au crucible can safely retain approximately 0.12 g of Hg. Remove the Hg from the Au crucible, preparatory to another experiment, by a short ignition at a dull red heat under a hood having a good draught. (The crucible will melt in the full heat of a Bunsen burner.)

*Precipitation Method*<sup>36</sup>—Official

127

## REAGENT

*Hydrogen peroxide soln.*—30%. Commonly designated as "perhydrol" or "super-oxol."

128

## DETERMINATION

Place 0.5–2.0 g of the sample, depending on the quantity of Hg present, in a 200 cc Erlenmeyer flask, fitted with an air condenser by means of a ground-glass joint. Add 10 cc of  $\text{H}_2\text{SO}_4$ , connect the flask to the condenser, and rotate in order to bring all the sample into contact with the acid. Then add dropwise thru the condenser tube 3–5 cc of the  $\text{H}_2\text{O}_2$  soln, and mix by rotation of the flask. After the active reaction has subsided, heat over a low flame for 15–20 min., add 5 cc more of the  $\text{H}_2\text{O}_2$ , and continue the heating until all organic matter is destroyed (indicated by a clear soln), adding more  $\text{H}_2\text{O}_2$  if necessary. Remove the flask from the heat, allow to cool, wash down the condenser, and transfer the contents to a beaker, filtering if necessary. Dilute to about 200 cc and destroy the excess of  $\text{H}_2\text{O}_2$  by titration with  $\text{KMnO}_4$  soln. Precipitate the Hg with  $\text{H}_2\text{S}$ , filter thru a weighed Gooch crucible, and dry the precipitate in the oven at 105–110°. Extract the dried precipitate with  $\text{CS}_2$  to remove any precipitated S, again dry, and weigh. From the weight of HgS calculate the percentage of metallic Hg, using the factor 0.86219.

**SODIUM HYPOCHLORITE SOLUTIONS**<sup>37</sup>

## SODIUM HYPOCHLORITE—OFFICIAL

*Arsenious Oxide Titration Method*

129

## REAGENTS

(a) *Arsenious oxide soln.*—0.1 N. Dissolve exactly 2.474 g of pure  $\text{As}_2\text{O}_3$  in a beaker by boiling with 150–200 cc of  $\text{H}_2\text{O}$  containing 10 cc of  $\text{H}_2\text{SO}_4$ . Cool, transfer to a 500 cc volumetric flask, and dilute to the mark.

(b) *Standard iodine soln.*—Prepare as directed under 3(b). Standardize against (a).

130

## DETERMINATION

Transfer a 20 cc aliquot of the sample to a liter volumetric flask and dilute to volume. Pipet a 50 cc aliquot of the mixture into a 200 cc Erlenmeyer flask. Add the standard  $\text{As}_2\text{O}_3$  soln in excess and then add a decided excess of  $\text{NaHCO}_3$ . Titrate the excess of  $\text{As}_2\text{O}_3$  with the standard I soln, using starch soln, 3(e), or the I as indicator. Subtract the volume of the I soln, corrected to 0.1 N, from the volume of  $\text{As}_2\text{O}_3$  soln used, and calculate the percentage of NaOCl. 1 cc of 0.1 N  $\text{As}_2\text{O}_3$  soln = 0.003723 g of NaOCl.

131

## AVAILABLE CHLORINE—OFFICIAL

Calculate the percentage of available chlorine from the titration described under 130. 1 cc of the 0.1 N  $\text{As}_2\text{O}_3$  = 0.003546 g of available Cl.

132

## CHLORIDE CHLORINE—OFFICIAL

Pipet a 50 cc aliquot of the prepared soln, 130, into a 200 cc Erlenmeyer flask and add a slight excess of the  $\text{As}_2\text{O}_3$  soln, 129(a), calculated from the NaOCl titration; add a slight excess of  $\text{HNO}_3$ , neutralize the soln with  $\text{CaCO}_3$ , and titrate with 0.1 N  $\text{AgNO}_3$ , 87, using  $\text{K}_2\text{CrO}_4$ , II, 52(b), or the Na arsenate formed in the soln

as indicator. Run a blank determination on the reagents and make correction for any Cl found. From this corrected titration and the sp. gr. of the sample calculate the percentage of Cl. From this value subtract one-half of the percentage of available Cl. The difference is the percentage of chloride chlorine.

## 133

## SODIUM HYDROXIDE—OFFICIAL

Pipet 25 cc of the sample into a 250 cc volumetric flask, and add sufficient  $\text{H}_2\text{O}_2$  soln that is neutral to phenolphthalein to destroy NaOCl. Mix well and add sufficient neutral 10%  $\text{BaCl}_2$  soln to precipitate the carbonates, make to volume, mix thoroly, and filter thru a dry filter. Pipet 50 cc of the filtrate into an Erlenmeyer flask and titrate with 0.1 N HCl, using phenolphthalein as indicator, 104(a). From this titration and the sp. gr. of the sample, calculate the percentage of NaOH.

## CARBON DIOXIDE—OFFICIAL

## 134

## APPARATUS

Connect an evolution flask, to which is attached a dropping funnel protected by a tube containing soda lime, to a condenser or a Kjeldahl distilling trap, which in turn is connected to two wash bottles containing 10% KI soln. Use glass beads or other device in the wash bottles to cause the gas to flow slowly thru the liquid. End the train with a Meyer absorption tube containing 0.1 N  $\text{Ba}(\text{OH})_2$  soln.

## 135

## DETERMINATION

Pipet a suitable aliquot of the sample (5–20 cc, governed by the quantity of  $\text{CO}_2$  present) into the evolution flask, and attach the flask to the train. Place 50 cc of 0.1 N  $\text{Ba}(\text{OH})_2$  soln in the Meyer tube, and add 35–50 cc of  $\text{H}_2\text{O}_2$  soln (or a sufficient quantity to reduce the hypochlorite) thru the dropping funnel into the evolution flask. After the action due to the  $\text{H}_2\text{O}_2$  has ceased, add 30 cc of HCl (1+3), heat the flask to boiling, and draw air slowly thru the apparatus. (The evolved gases will be freed from Cl by the KI in the wash bottles, and the  $\text{CO}_2$  will be absorbed in the standard  $\text{Ba}(\text{OH})_2$  in the Meyer tube.) Draw the air thru the apparatus for 20 min., disconnect the Meyer tube, and pour its contents into a beaker. Wash out the tube, adding the washings to the contents of the beaker. Filter, wash, and titrate the filtrate and washings with 0.1 N HCl, using phenolphthalein as indicator, 104(a). From the number of cc of  $\text{Ba}(\text{OH})_2$  used and the sp. gr. of the sample, calculate the percentage of  $\text{CO}_2$ . 1 cc of 0.1 N  $\text{Ba}(\text{OH})_2$  = 0.00220 g of  $\text{CO}_2$ .

CALCIUM HYPOCHLORITE AND BLEACHING POWDER<sup>38</sup>

## AVAILABLE CHLORINE—OFFICIAL

## 136

*Arsenious Oxide Titration Method*

Weigh 5–10 g of the thoroly mixed sample into a porcelain mortar, add 30–40 cc of  $\text{H}_2\text{O}$ , and triturate until a smooth cream is obtained (in the case of high-test  $\text{Ca}(\text{OCl})_2$ , the sample will dissolve readily and will not form a cream.) Add more  $\text{H}_2\text{O}$ , stir well with the pestle, and allow the insoluble residue to settle for a few moments. Pour the mixture off into a liter volumetric flask, add more  $\text{H}_2\text{O}$ , and thoroly triturate the sample and pour off as before. Repeat the operation until all the material has been transferred to the flask. Rinse the mortar and pestle, catch the wash  $\text{H}_2\text{O}$  in the flask, dilute the soln to the mark, and mix. Without allowing the material



to settle, pipet a 25–50 cc aliquot into a 200 cc Erlenmeyer flask. Add the standard  $\text{As}_2\text{O}_3$  soln, 129(a), in excess and then add a decided excess of  $\text{NaHCO}_3$ . Titrate the excess of  $\text{As}_2\text{O}_3$  with the standard I soln, 129(b), using starch soln, 3(e), or the I as indicator. Subtract the volume of the I soln, corrected to 0.1 *N*, from the volume of  $\text{As}_2\text{O}_3$  soln used, and calculate the percentage of available Cl. 1 cc of 0.1 *N*  $\text{As}_2\text{O}_3$  = 0.003546 g of available Cl.

### CHLORAMINE-T<sup>38</sup>

#### ACTIVE CHLORINE—OFFICIAL

##### *Arsenious Oxide Titration Method*

137

#### REAGENTS

Use the reagents described under 129.

138

#### DETERMINATION

Transfer 0.5 g of the sample to a 300–500 cc Erlenmeyer flask, dissolve in 50 cc of  $\text{H}_2\text{O}$ , and add an excess of the standard  $\text{As}_2\text{O}_3$  soln, 129(a), and 5 cc of  $\text{H}_2\text{SO}_4$  (1+4). Add a decided excess of  $\text{NaHCO}_3$  and titrate the excess  $\text{As}_2\text{O}_3$  with standard I soln, 129(b), using starch soln, 3(e), or the I as indicator. From this titration calculate the active Cl in the sample. 1 cc of 0.1 *N*  $\text{As}_2\text{O}_3$  soln = 0.001773 g of active Cl.

139

#### TOTAL CHLORINE—OFFICIAL

Dissolve 0.5 g of the sample in 50 cc of  $\text{H}_2\text{O}$  in an Erlenmeyer flask and add a slight excess of the standard  $\text{As}_2\text{O}_3$  soln calculated from the active Cl titration, 138. Add 5 cc of  $\text{HNO}_3$  (1+4), neutralize with  $\text{CaCO}_3$ , and titrate with standard  $\text{AgNO}_3$ , 87, using  $\text{K}_2\text{CrO}_4$ , II, 52(b), as indicator. Run a blank titration on the reagents and make correction for any Cl found. From the corrected titration calculate the percentage of total Cl in the sample. 1 cc of 0.1 *N*  $\text{AgNO}_3$  = 0.003546 g of Cl. If the total Cl exceeds the active Cl, the presence of NaCl is indicated.

140

#### SODIUM—OFFICIAL

Weigh 0.5 g of the sample in a silica or porcelain dish and add about 25 cc of  $\text{H}_2\text{O}$  and 3–5 cc of  $\text{H}_2\text{SO}_4$  (1+4). Evaporate to a sirupy consistency on a steam bath and finally to dryness on a hot plate. Ignite at the full heat of a Bunsen burner, cool, and weigh as  $\text{Na}_2\text{SO}_4$ . (The residue should be completely soluble in  $\text{H}_2\text{O}$  and should show no turbidity with  $\text{NH}_4\text{OH}$  and  $(\text{NH}_4)_2\text{CO}_3$ .) Test with a flame for Na. If the residue meets these tests it may be considered pure  $\text{Na}_2\text{SO}_4$ . From the weight of the residue calculate the percentage of Na in the sample.

### PHENOL COEFFICIENT<sup>39</sup> TENTATIVE

141

#### I. USING EBERTHELLA TYPHOSA

(Applicable to the testing of coal tar disinfectants that are miscible with  $\text{H}_2\text{O}$  and to other disinfectants that are miscible with  $\text{H}_2\text{O}$  and act against bacteria in a manner similar to phenol. False values are obtained from certain products that are highly inhibitory, such as mercury compounds, and the values obtained from testing oxidizing products may be highly misleading.)

142

#### REAGENTS

(a) *Culture media*. (1) *Nutrient broth*: Boil 5 g of Liebig's beef extract, 5 g of NaCl, and 10 g of Armour's peptone (quality specially prepared for disinfectant testing) in 1000 cc of  $\text{H}_2\text{O}$  for 20 min., make up to volume with  $\text{H}_2\text{O}$ , and adjust to pH 6.8 (using a colorimetric method, adjust the broth to give a dark green color

with bromothymol blue). Filter thru paper, place 10 cc amounts in 20×150 mm bacteriological test tubes, plug with cotton, and sterilize at 15 lbs. pressure for 40 min. Use this broth for daily transfers and for subcultures. (2) *Nutrient agar*: Dissolve 1.5% Bacto agar (Difco) in nutrient broth and adjust to pH 7.2-7.4 (which gives a blue-green color with bromothymol blue), tube, plug with cotton, sterilize, and slant.

(b) *Test organism*.—The Hopkins strain of *Eberthella typhosa* (Zopf) Weldin (frequently called *Bac. typhosus*). Carry a stock culture on nutrient agar slants. Transfer once a month and incubate new stock transfer for 2 days at 37°, then store at room temp.

From the stock culture inoculate a tube of nutrient broth and make at least 4 consecutive daily transfers (not over 30) in nutrient broth, incubating at 37°, before using the culture for testing (if only 1 daily transfer has been missed it is not necessary to repeat the 4 consecutive transfers). Use a 22-26 hours' culture of the organism grown in nutrient broth at 37° in the test. Shake, and allow to settle 15 min. before using.

(c) *Phenol*.<sup>40</sup>—Use phenol that meets the requirements of the U.S.P. and has a congealing point 40° or above. Use 5% soln as a stock soln and keep in well-stoppered amber bottles in a relatively cool place, protected from light. Standardize with 0.1 N bromine or Na bromide and bromate soln.<sup>41</sup>

#### 143

##### APPARATUS

(a) *Glassware*.—1, 5, and 10 cc volumetric pipets; 1, 5, and 10 cc Mohr. pipets graduated to 0.1 cc or less; 100 cc stoppered cylinders graduated in 1 cc divisions. Pyrex lipped test tubes 25×150 mm. Plug the test tubes (medication tubes) with cotton wrapped in 1 layer of cheese cloth. Sterilize all glassware in a hot air oven at 180° for 2 hours. Place pipets in closed metal containers before sterilizing.

(b) *Water bath*.—An insulated relatively deep water bath with a cover having at least 10 well-spaced holes which admit the medication tubes but not their lips.

(c) *Racks*.—May be of any convenient style. Blocks of wood (size depending somewhat on the incubator to accommodate them) with deep holes are satisfactory. Have the holes well-spaced to insure quick manipulation of the tubes; and it is convenient to have them large enough to admit the medication tubes while dilutions are being made.

(d) *Transfer loop*.—Make a 4 mm (inside diameter) single loop at the end of a No. 23 B & S Gage Pt. wire 2 3 in. long, and have the other end in a suitable holder (glass or aluminum rod). Bend the loop at a 30° angle with the stem (Fig. 9).

#### 144

##### PROCEDURE

Make a 1% stock dilution of the substance to be tested (or any other convenient dilution, depending on the anticipated strength) in a glass-stoppered cylinder. Make the final dilutions, from the 1% stock dilution, directly into the medication tubes and remove all excess over 5 cc. (The range of dilutions should cover the killing limits of the disinfectant within 5 and 15 min. periods and should at the same time be sufficiently close for accuracy.) From the 5% stock soln make a 1-90 and a 1-100 dilution of the phenol directly into medication tubes. Place these tubes, containing 5 cc each of the final dilutions of disinfectant and of phenol, in the water bath at 20° and leave for 5 min. Add 0.5 cc of the test culture to each of the dilutions at time intervals corresponding to the intervals at which the transfers are to be made. (Thus, by the time 10 tubes have been seeded at 30 second intervals, 4.5 min. will have elapsed, and a 30 second interval intervenes before the transference to the sub-cultures is commenced.) Add the culture from a graduated pipet of

sufficient capacity to seed all the tubes in any one set. (As a precautionary measure the pipet should be loosely plugged with cotton at the mouth end before being sterilized. The temp. of the culture should be practically that of the water bath before it is added.)

In inoculating the medication tubes, hold them in a slanting position after removal from the bath, insert the pipet to just above the surface of the disinfectant, and run in the culture without allowing the tip to touch the disinfectant. After adding the culture, agitate the tubes gently but thoroly to insure even distribution of the bacteria, and replace in the bath; 5 min. after seeding the first medication tube, transfer 1 loopful of the mixture of culture and diluted disinfectant from the medication tube to the corresponding sub-culture tube. To facilitate the transfer of uniform drops of the medication mixture, hold the tube at a 60° angle,

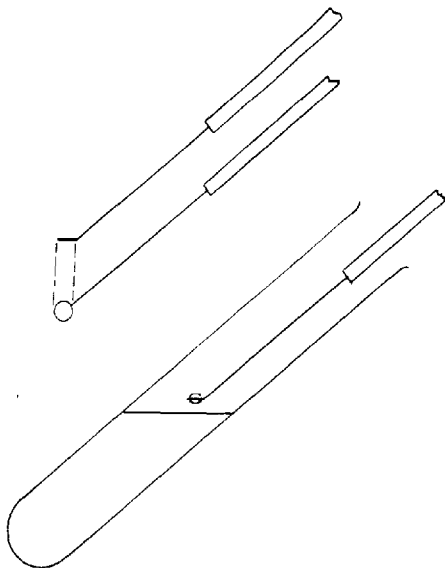


FIG. 9.—TRANSFER LOOP AND MANNER OF USING IN DETERMINATION OF PHENOL COEFFICIENT

and withdraw the loop so that the plane of the loop is parallel with the surface of the liquid (see Fig. 9). At the end of 30 seconds, transfer a loopful from the second medication tube to the second sub-culture tube and continue the process for each successive dilution; 5 min. after making the first transfer, begin a second set of transfers for the 10 min. period, and finally repeat for the 15 min. period. Before each transfer heat the loop to redness in the Bunsen flame and flame the mouth of every tube. Sterilize the loop immediately after each transfer (before replugging the tubes) to allow time for cooling. Use care in transferring and seeding to prevent the pipet or needle from touching the sides or mouth of the medication tube and see that no cotton threads adhere to the inner sides or the mouth of the tubes. Incubate the sub-cultures at 37° for 48 hours and read results. Macroscopic examination is usually sufficient. Occasionally a 3-day incubation period, an agar streak, a microscopical examination, or agglutination with antityphoid serum may be necessary to determine feeble growth or suspected contamination.

145

## CALCULATION

Express the results in terms of the phenol coefficient, a number obtained by dividing the numerical value of the greatest dilution (the denominator of the fraction expressing the dilution) of the disinfectant capable of killing *Eb. typhosa* in 10 min. but not in 5 min. by the greatest dilution of phenol showing the same results.

Example:

	5 MIN.	10 MIN.	15 MIN.
Disinfectant (X):			
1-300	0	0	0
1-325	+	0	0
1-350	+	0	0
1-375	+	+	0
1-400	+	+	+
Phenol:			
1-90	+	0	0
1-100	+	+	+

Phenol coefficient would be  $\frac{350}{90} = 3.89$ .

The test is satisfactory only when the phenol control gives one of the following readings:

PHENOL	5 MIN.	10 MIN.	15 MIN.
1-90	+ or 0	+ or 0	0
1-100	+	+	+ or 0

If none of the dilutions of the disinfectant shows growth in 5 min. and killing in 10 min., estimate the hypothetical dilution only when any 3 consecutive dilutions show the following results: The first, no growth in 5 min.; the second, growth in 5 and 10 min. but not in 15 min.; and the third, growth in 5, 10, and 15 min.

Example:

	5 MIN.	10 MIN.	15 MIN.
Disinfectant (X):			
1-300	0	0	0
1-350	+	+	0
1-400	+	+	+
Phenol:			
1-90	0	0	0
1-100	+	+	0

Phenol coefficient would be  $\frac{325}{95} = 3.42$ .

To avoid giving an impression of fictitious accuracy, calculate the phenol coefficient to the nearest 0.1. Thus, in the examples cited above, the phenol coefficients would be reported as 3.9 and 3.4, instead of 3.89 and 3.42.

NOTE.—The commonly accepted criterion that disinfectants for general use be employed at a dilution equivalent in germicidal efficiency to 5% phenol against *Eb. typhosa* (that is, 20 times the *Eb. typhosa* coefficient) allows a reasonable margin of safety for the destruction of infective agents likely to be the object of general disinfection.

146

II. USING STAPHYLOCOCCUS AUREUS<sup>a</sup>

(Applicable in the bacteriological examination of disinfectants to be used for special purposes, such as the disinfection of dental and surgical or veterinary instruments.)

Proceed as directed above except to change the phenol dilutions. Use a temp. of 20° unless otherwise directed. The culture of *Staph. aureus* must have at least the resistance indicated by the following:

AT 20°	PHENOL	5 MIN.	10 MIN.	15 MIN.
	1-60	+	0	0
	1-70	+	+	+

The resistance of the culture to phenol when used at 37° must be as follows:

PHENOL	5 MIN.	10 MIN.	15 MIN.
1-80	+	0	0
1-90	+	+	+ or 0

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## VII. CAUSTIC POISONS

### PHENOL

#### *Method I—Official*

(Applicable to the determination of phenol in commercial cresols, saponified cresol solns, coal tar dips, and disinfectants, and to kerosene solns of phenols except in the presence of salicylates or betanaphthol.)

#### 1

##### REAGENTS

(a) *Dilute nitric acid*.—Blow air thru  $\text{HNO}_3$  until it is colorless and dilute 1 volume of this acid with 4 volumes of  $\text{H}_2\text{O}$ .

(b) *Millon's reagent*.—Treat 2 cc of Hg in a 200 cc Erlenmeyer flask with 20 cc of  $\text{HNO}_3$ . Place the flask under a hood, and after the first violent reaction is over shake as much as necessary to effect subdivision of the Hg and maintain action. After about 10 min., when the action has practically ceased even in the presence of undissolved Hg, add 35 cc of  $\text{H}_2\text{O}$ , and if basic salt separates, add a sufficient quantity of the dilute  $\text{HNO}_3$  to dissolve it. Add a 10% soln of NaOH dropwise with thorough mixing until the curdy precipitate that forms after the addition of each drop no longer redissolves but disperses to an evidently permanent turbidity. Then add 5 cc of the dilute  $\text{HNO}_3$  and mix well. As the soln deteriorates, do not use it after the first day.

(c) *Standard phenol soln*.—Dissolve a weighed quantity of the pure substance (congealing point not lower than  $40^\circ$ ) in a sufficient quantity of  $\text{H}_2\text{O}$  to make not less than a 1% soln. On the day it is to be used, from this stock soln make a 0.025% soln (the final standard) in additional distilled  $\text{H}_2\text{O}$ .

(d) *Formaldehyde soln*.—Dilute 2 cc of commercial 37%  $\text{HCHO}$  soln to 100 cc with distilled  $\text{H}_2\text{O}$ .

#### 2

##### APPARATUS

(a) *Nessler cylinders*.—50 cc tall form, matched.

(b) *Test tubes*.—Approximately 180 mm  $\times$  20 mm, provided with rubber stoppers and marked at 25 cc.

(c) *Water bath for heating the test tubes*.—A beaker containing a disk of wire gauze raised about an inch from the bottom may be used.

#### 3

##### PREPARATION OF SAMPLE

(a) *Commercial cresol*.—Weigh by difference about 2.5 g of sample into a 250 cc volumetric flask, dissolve in 10 cc of a 10% NaOH soln, and make to the mark with  $\text{H}_2\text{O}$ .

(b) *Saponified cresol solns, coal tar dips and disinfectants, kerosene solns of phenols, etc.*—Weigh by difference about 5 g (or use 5 cc and calculate the weight from the density of the sample) of sample into a 250 cc volumetric flask and dilute to the mark with  $\text{H}_2\text{O}$ . In products consisting largely of kerosene, bring the  $\text{H}_2\text{O}$  level to the mark and take aliquots from the aqueous portion only.

#### 4

##### DETERMINATION

Transfer a 5 cc aliquot of the prepared soln to a 200 cc volumetric flask shortly before the determination is to be carried out, dilute to about 50 cc, add 1 drop of

methyl orange indicator, VI, 3(f), and then the dilute  $\text{HNO}_3$  until the soln is practically neutral, make to volume, and shake well.

Place 5 cc of the diluted soln in each of 2 of the marked test tubes, and in each of 2 additional test tubes place 5 cc of the standard phenol soln. Next flow 5 cc of the Millon's reagent down the side of each tube, mix, and place the tubes in a bath of boiling  $\text{H}_2\text{O}$ ; continue the boiling for exactly 30 min.; cool immediately and thoroly by immersion in a bath of cold  $\text{H}_2\text{O}$  for at least 10 min., and add 5 cc of the dilute  $\text{HNO}_3$  to each tube.

Mix well and add 3 cc of the dilute  $\text{HCHO}$  soln to one of each pair of tubes; make all the tubes to the 25 cc mark with  $\text{H}_2\text{O}$ , stopper, shake well, and allow to stand overnight. The next day the contents of the tubes to which  $\text{HCHO}$  was added will have faded to a yellow, while the others will show an orange or red tint.

Pipet 20 cc from each of the 2 phenol tubes and transfer to 100 cc volumetric flasks; treat each with 5 cc of the dilute  $\text{HNO}_3$ , make to the mark, and mix. The red flask contains the "phenol standard," and the yellow flask the "phenol blank." Transfer these solns to burets. Pipet 10 cc of each sample soln into Nessler tubes. (The orange or red constitutes the "unknown" and the yellow the "sample blank," and each Nessler tube must be distinctly marked to avoid confusion.) Next add to the "sample blank" tube a measured quantity of "phenol standard" and add the same volume of "phenol blank" to the "unknown," thoroly agitate (aided by insertion of the rubber stoppers if necessary), and compare the colors. When the tubes have been brought to a match, each cc of the phenol standard used = 1% of phenol if a portion of sample weighing exactly 5 g was used, or 2% if exactly 2.5 g was used.

NOTE.—In using this method the following precautions should be borne in mind: A pair of phenol tubes affords sufficient final solns for assaying several unknowns, but all the latter must have accompanied the phenol solns thruout the entire process with identical reagents and treatment. If the end point has been inadvertently overrun it is possible to work back to it, but since mistakes are easy to make in this procedure it is better to repeat the comparison on fresh portions from the original tubes. Too much delay in matching the tubes must be avoided after the titration has been started, otherwise the excess of  $\text{HCHO}$  present in the blanks may have time after mixture to affect the intensity of the red color.

Millon's reagent is dangerously poisonous and should not be transferred with an ordinary pipet and mouth suction unless a protective trap of some kind is used.

5

#### *Method 11<sup>2</sup>—Official*

(Applicable to the determination of phenol in the presence of salicylates.)

Weigh by difference into a separatory funnel approximately 10 g of sample (or use 10 cc and calculate the weight from the density of the sample). Add 50 cc of kerosene and extract 3 times with 100 cc portions of  $\text{H}_2\text{O}$ . Filter the aqueous extracts thru a wet filter into a 500 cc volumetric flask, make to volume with distilled  $\text{H}_2\text{O}$ , and proceed as directed under 4.

When the tubes have been brought to a match, each cc of the phenol standard used = 1% of phenol if a portion of the sample weighing exactly 10 g was used.

#### SELECTED REFERENCES

- <sup>1</sup> U. S. Dept. Agr. Bull., 1308, p. 17; J. Assoc. Official Agr. Chem., 13, 49 (1930).  
<sup>2</sup> Ind. Eng. Chem. Anal. Ed., 1, 232 (1929).

## VIII. NAVAL STORES

### ROSIN

#### ANALYTICAL METHODS

1

##### SAMPLING—TENTATIVE

Remove the top 6 inches of the rosin in the barrel or drum, and by means of a sharp spike take a single lump of about 1 lb. from a depth of 6-8 in. below the original surface of the rosin in the barrel or drum. Do not break up or pulverize this lump. When acid number, saponification number, unsaponifiable matter, petroleum ether insoluble or ash is to be determined obtain the stated quantity by breaking off small pieces having freshly exposed surfaces, avoiding powdering as much as possible. (These precautions are necessary because rosin oxidizes rapidly on the surface when exposed to the air.)

2

##### ACID NUMBER—TENTATIVE

To 2 g of the rosin in a 300 ml Erlenmeyer flask, add 50 ml of neutral 95% ethyl alcohol. Allow the rosin to dissolve in the alcohol at normal temp., or with the aid of heat, and cool to room temp. Titrate with 0.5 N NaOH soln, using phenolphthalein, II, 10(d), as indicator. If necessary, in order to obtain a sharp end point, dilute the solns of the redder rosins with additional neutral alcohol. Calculate as the acid number the mg of KOH required to neutralize 1 g of the rosin.

3

##### ACID NUMBER OF DARK COLORED ROSIN—TENTATIVE

With low-grade dark colored rosin, where the color of the soln interferes with observation of the end point, use the following method based on the use of a small direct vision hand spectroscope to observe the appearance of an absorption band in the green part of the spectrum on liberation of the alkaline phenolphthalein color body.<sup>1</sup>

Place 100 cc of 95% alcohol and 1 cc of phenolphthalein indicator soln in a 300 cc Erlenmeyer flask. Add 0.5 N NaOH (1 or 2 drops should suffice) until the absorption band appears when viewing the spectrum through a 1 in. (2.5 cm) depth of liquid. Introduce 5 g of the rosin, previously weighed, into the flask, stopper, and allow to dissolve at room temp. Titrate with the 0.5 N NaOH, running in about 1 cc less than the expected or theoretical quantity required for the weight of sample taken. After the further addition of each 0.05 or 0.10 cc of alkali, hold the flask in an inclined position towards a source of light, preferably daylight, and observe the spectrum through a depth of about 1 in. The end point is reached when the absorption band similar to that previously observed again becomes just perceptible.

4

##### SAPONIFICATION NUMBER—TENTATIVE

Weigh accurately 2 g of the rosin sample into a 300 cc Erlenmeyer flask. Add 50 cc of neutral 95% alcohol and 20 cc of alcoholic KOH soln, XXXI, 22, allowing the pipet to drain for a definite time. Connect the flask to a reflux condenser, heat on a steam bath for 1 hour, cool, and titrate with 0.5 N HCl, II, 19(a), using phenolphthalein as indicator. Dilute the soln if necessary with neutral 95% alcohol in order to obtain a sharp end point. If the dark color of the soln prevents observation of the end point, use the spectroscope method described under 3. The dis-



appearance from the spectrum of the characteristic absorption band marks the end point in this instance. This is reached when the addition of 1 or 2 drops of the acid causes the disappearance and the addition of a similar quantity of alkali brings back the band. Conduct a blank determination, using the same pipet for measuring the KOH soln and drain for the same length of time. Subtract the number of cc of the 0.5 *N* HCl obtained in the determination on the sample from the number obtained on the blank to obtain the number of cc of 0.5 *N* HCl equivalent to the KOH used in the saponification of the sample taken. Calculate as saponification number the mg of KOH required to saponify 1 g of rosin.

#### TOLUOL-INSOLUBLE MATERIAL<sup>2</sup>—TENTATIVE

5

##### PREPARATION OF SAMPLE

(1) If the sample is less than 200 g, immediately before making the determination powder it to pass a standard 10-mesh sieve, mix thoroly, and place in a wide-mouthed bottle of such size that the sample completely fills it.

(2) If the sample is more than 200 g, crush it to pass a  $\frac{1}{4}$ -in. sieve, mix, quarter down to about 200 g, and treat as described in (1).

6

##### PROCEDURE

Place 50 g of the freshly-powdered sample in a 300 cc beaker, add 150 cc of toluol free from H<sub>2</sub>O and non-volatile residue, and dissolve the sample with the aid of heat and occasional shaking. When the soln is apparently complete (no particles of rosin visible), filter at once thru a 25 cc porcelain Gooch crucible which has been previously prepared with a mat of pure, well-washed asbestos and which has been finally washed thoroly with the solvent used, dried in a boiling-water oven for 30 min., cooled in a desiccator, and weighed. If the rosin filtrate is not clear, return it thru the Gooch crucible until it is clear, finally washing the residue and the outside of the crucible free from rosin with additional hot solvent. Dry the crucible and contents to constant weight at 105-110° in an oven (1 hour usually suffices), cool in a desiccator, weigh, and calculate the percentage of toluol insoluble.

7

#### PETROLEUM ETHER-INSOLUBLE MATTER (OXIDIZED ROSIN) -TENTATIVE

Weigh 1 g of freshly pulverized rosin into a tared 250 cc glass-stoppered (a cork covered with tin foil may be used) Erlenmeyer flask. Add 100 cc of petroleum ether (boiling point 30-75°), stopper the flask, and shake to prevent the rosin coalescing or adhering to the flask. Add an additional 50 cc of petroleum ether, stopper, and shake vigorously for about 5 min., or until any undissolved rosin is in a finely divided state and does not adhere to the flask. Allow the flask to stand overnight at a temp. of 23-28°, and filter the soln through a tared Gooch crucible, rinsing the flask with about 50 cc of petroleum ether. Wipe the outside of the flask and crucible with a cloth wet with alcohol or acetone and dry in an oven at 95-100° for 1.5 hours. Cool, and weigh. From the combined weights of the residue in the flask and in the crucible calculate the percentage of petroleum ether-insoluble matter.

This method determines the degree of oxidation. If the rosin is appreciably "dirty," determine the extraneous matter (toluol-insoluble matter) according to 5 and 6, and subtract the quantity found from the total quantity of petroleum ether-insoluble.

8

#### ASH -TENTATIVE

Weigh 10 g of rosin into a porcelain crucible, burn off the combustible matter

slowly, and ignite the residue until the ash is free from carbonaceous matter. Cool the crucible in a desiccator and weigh. Report the result as percentage of ash.

9

## VOLATILE OILS—TENTATIVE

Place 100 or 200 g of freshly broken lumps of rosin in a liter distilling flask; set the flask in an oil bath maintained at 160–170°; and distil with steam, receiving the distillate in a graduated cylinder. Measure exactly the volume of separated oil and report as cc per 100 g of the rosin.

## ROSIN GRADING

10

## SAMPLING—TENTATIVE

Take a sample by any of the following procedures:

(a) Remove by spiking with a pointed heavy iron bar (1) a lump of rosin roughly 4–6 inches in diameter (2) from 6–8 inches below the surface of the rosin in the barrel or drum. With a special rosin adz (3) or sampling hatchet (4) cut a cube with parallel sides exactly  $\frac{3}{4}$  inch apart in one direction (5).

(b) Immediately after the barrel is filled, suspend in the rosin in a horizontal position a tubular mold  $\frac{3}{4}$  inch square (inside) (6), 1½ inches or more in length, made of thin, well-tinned plate. Place the mold sufficiently deep (at least 6 inches in hot rosin) to insure a depth of at least 4 inches below the surface of the rosin after it solidifies. After the rosin has thoroly cooled, remove the sample (7) by spiking as directed in (a).

(c) Remove in the manner devised for the particular type of sampler (8) the sample (9) contained in a mold (8) made of well-tinned plate (which mold was placed in the barrel or drum before it was filled with hot rosin) through an opening, the top of which is 8 inches from the top of the barrel or drum. The mold thus placed must be entirely within the barrel or drum and completely encased in the rosin.

If any sample as prepared for grading is too large, so that it cannot be viewed through a thickness of exactly  $\frac{3}{4}$  inch, or has irregular uneven surfaces, bring it to the correct size by smoothing against a hot “smoothing” iron (10) or hot flat iron, taking care to wipe off any adhering hot rosin remaining from a previous application.

11

## DETERMINATION

Compare the sample obtained as directed in (a), (b), or (c) with a set of duplicates of the United States Rosin Standards (11) or with a set of type samples or “types” (12), made of rosin, which match the United States Rosin Standards, either with or without the comparison box (13). To be of a given grade the sample of rosin must be equal to or better, that is, lighter in color than the standard for that grade. If the grader cannot decide whether the sample is equal to a standard or is darker than the standard for a grade, it is given that grade. For example, if a sample is being compared with the “N” standard, and the grader cannot definitely decide whether the sample is equal to or is darker than the standard the sample should be graded “N.”

TURPENTINE OIL<sup>2</sup> (SPIRITS OF TURPENTINE)

12

## COLOR—TENTATIVE

Place a 200 cc flat-bottomed colorimeter tube graduated in mm and filled to a depth of 40–50 mm with the turpentine in a colorimeter and on or under it place a No. 2 yellow Lovibond glass. Over or under a second graduated tube in the colorim-

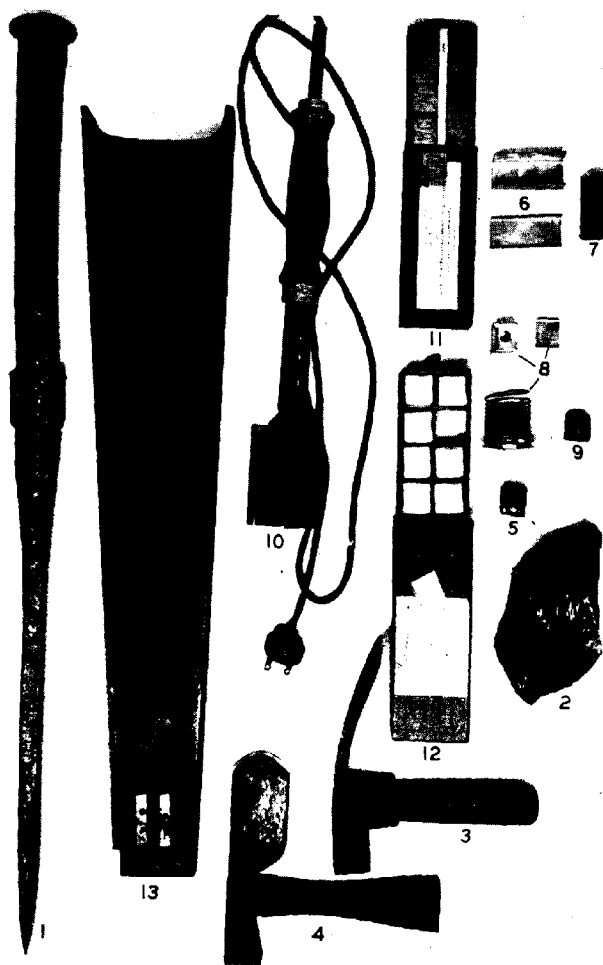


FIG. 10.—ARTICLES USED IN GRADING ROSIN

- |                          |  |
|--------------------------|--|
| 1. Spike.                | 8. Short mold.                         |
| 2. Rosin lump.           | 9. Sample taken with it.               |
| 3. Adz.                  | 10. Smoothing iron.                    |
| 4. Hatchet.              | 11. Duplicates, U. S. Rosin Standards. |
| 5. Cut sample rosin.     | 12. Rosin types.                       |
| 6. Long mold.            | 13. Comparison box.                    |
| 7. Sample taken with it. |  |

eter, place a No. 1 yellow Lovibond glass and run into it the same turpentine until the color matches as nearly as possible the color in the first tube. Read the difference in depth of the turpentine in the 2 tubes. If this difference is 50 to 149.9 mm, the turpentine is "standard"; if it is 150 mm or more, the turpentine is "water-white"; and if the difference is from 25 to 49.9 mm, the turpentine is "one shade off."

## 13

## SPECIFIC GRAVITY—TENTATIVE

Determine the specific gravity at 15.5/15.5° by any convenient method that is accurate within 2 points in the fourth place. If the determination is made at any

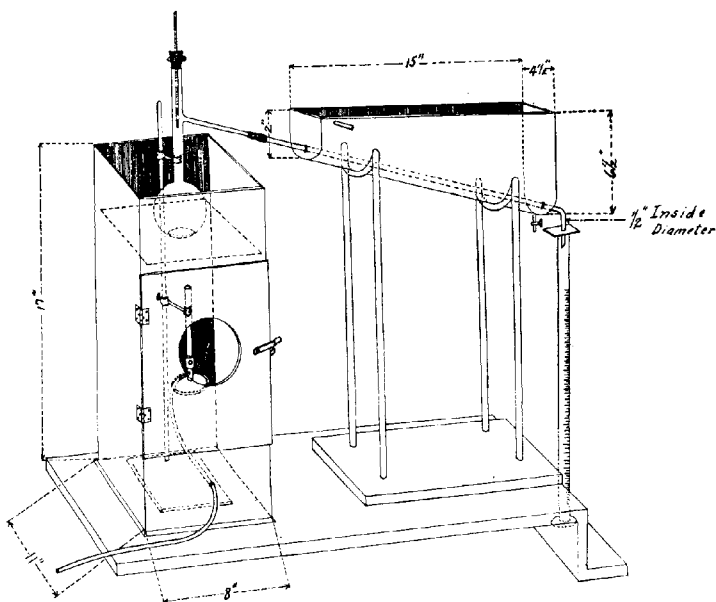


FIG. 11.—APPARATUS FOR DISTILLATION OF TURPENTINE OIL  
OVER OPEN FLAME

other temp., correct the reading by adding thereto or subtracting therefrom 0.00082 for each degree that the temp. at which the determination is made is respectively above or below 15.5°.

## 14

## REFRACTIVE INDEX—TENTATIVE

Determine the refractive index at any convenient temp., but preferably at 20°. If determined at other than 20°, calculate the result to 20° by adding or subtracting the correction factor 0.00045 for each degree that the temp. of the determination is above or below 20°, respectively.

## DISTILLATION—TENTATIVE

15

## APPARATUS

(a) *Flask*.—Use an Engler flask having the following dimensions: Diameter of bulb, 6.5 cm; cylindrical neck, 15 cm long, 1.6 cm internal diameter; side or vapor tube, 10 cm long, 0.6 cm external diameter, attached to neck at an angle of  $75^\circ$ , so that when the flask contains its charge of 100 cc of oil the surface of the oil shall be 9 cm below the bottom of the junction of the side tube and neck.

Support the flask on a plate of asbestos 20 cm square, having an opening 4 cm in diameter in its center, and heat with an open flame; or support the flask in a metal cup, 15–20 cm in diameter, containing high boiling mineral oil or glycerol and fitted with a concave cover having in the center a circular opening  $5\frac{1}{2}$ –6 cm in diameter (Fig. 11). Surround the flask and burner with a shield to prevent fluctuation in temp. in the neck of the flask.

(b) *Condenser*.—(1) Use the form<sup>1</sup> illustrated in Fig. 11, which consists of thin-walled brass condenser tubing (No. 20 Stubbs gage seamless)  $\frac{1}{2}$  in. inside diameter and 22 in. long, placed at an angle of  $75^\circ$  in a metal cooling bath of the size and dimensions shown in Fig. 11. The lower end of the condenser is cut off at an acute angle and curved down for a length of 3 in. so as to project at least  $\frac{1}{2}$  in. into the receiving cylinder; or, (2) use a straight glass condenser 22 in. long, having 16 in. in contact with the cooling  $H_2O$  and fitted with an adapter, the small end of which, cut off at an acute angle, is long enough to extend a short distance into the receiving cylinder as illustrated in Fig. 12.

(c) *Thermometer*.—Use an accurate thermometer of the Anschütz type, conforming to the following specifications: Graduated from  $145$  to  $200^\circ$  in  $0.2^\circ$  intervals. Length, bottom of thermometer to  $175^\circ$  mark, not more than 8 nor less than 6.5 cm; top of bulb to  $145^\circ$  mark, not less than 1.5 cm; from  $145$  to  $175^\circ$  mark, not more than 6 cm. The graduation marks and the numbering shall be clear-cut and distinct. The error at any point on the scale shall not exceed  $\pm 0.5^\circ$  when tested for total immersion of the Hg column.

(d) *Receiving cylinder*.—Use an accurately graduated 50–100 cc cylinder. The so-called normal or precision cylinder of 50 cc capacity, having an internal diameter of 1.5 cm and graduated in 0.2 cc, is preferred. If a cylinder with larger inside diameter is used, place over the top a pasteboard cover having an opening for the condenser tube.

16

## DETERMINATION

Place 100 cc of the turpentine and several small pieces of pumice (or glass) in the distilling flask. Fit the thermometer so that the top of the Hg bulb is level with the bottom of the side tube and the  $175^\circ$  mark is below the cork. Place the flask in position on the asbestos board or oil bath and connect with the condenser. Apply the heat cautiously at first, and when distillation begins so regulate that the turpentine distils at the rate of not less than 4 nor more than 5 cc per min. (approximately 2 drops per second). The initial boiling point is the thermometer reading at the instant when the first drop falls from the end of the condenser. Discontinue distillation when the temp. reaches  $170.0^\circ$ , or the equivalent thereof, depending on the atmospheric pressure, as determined under 17. Let the condenser drain and read the percentage distilled. The percentage distilled below successive selected temps. and the temp. at which each successive 10 cc distils may also be determined, if desired, the necessary correction of the temp. being made for variations in atmospheric pressure.

## 17 CORRECTION FOR VARIATION IN ATMOSPHERIC PRESSURE—TENTATIVE

The distilling temp. of turpentine is affected  $0.057^{\circ}$  for each mm variation in barometric pressure. If the barometer reading after correcting to  $0^{\circ}$  is above or below the normal 760 mm, the turpentine will distil at a higher or lower temp., respectively, than at normal pressure. Therefore, for each mm that the corrected barometer reading is above 760 mm, correct the initial boiling-point reading by minus ( $-$ )  $0.057^{\circ}$ ; and for each mm that the corrected barometer reading is below

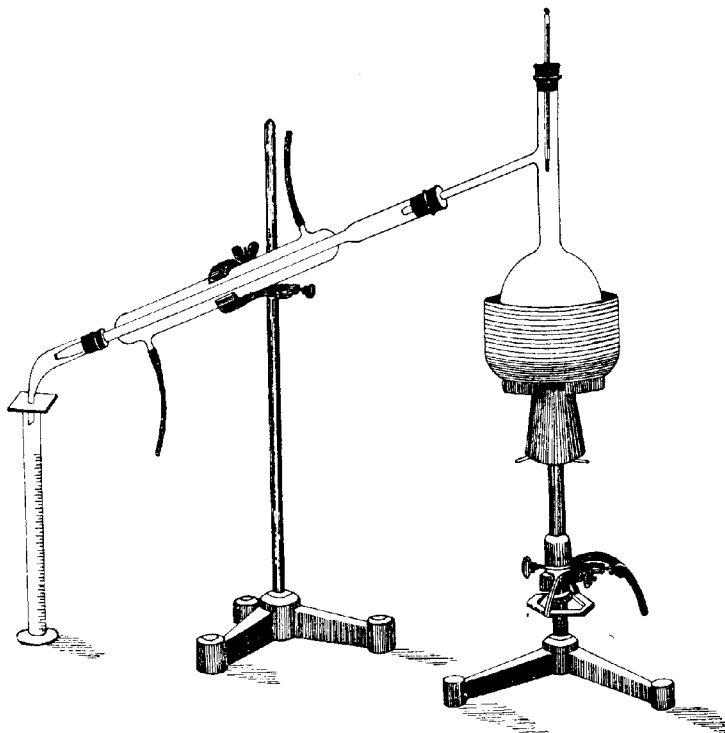


FIG. 12.—APPARATUS FOR THE DISTILLATION OF TURPENTINE OIL OVER BATH

760 mm, correct the initial boiling-point reading by plus (+)  $0.057^{\circ}$ . Also correct the final temp. observation point ( $170^{\circ}$ ) in the same way, by adding thereto  $0.057^{\circ}$  for each mm of pressure above 760 mm, or subtracting therefrom  $0.057^{\circ}$  for each mm of pressure below 760 mm, as may be required. The actual temp. at which distillation is stopped must be that equivalent to  $170^{\circ}$  at 760 mm.

## MINERAL OIL IN TURPENTINE

*Fuming Sulfuric Acid Method*<sup>s</sup>—Official

## 18

## REAGENT

*Fuming 38 N sulfuric acid.*—Mix  $\text{H}_2\text{SO}_4$  with sufficient fuming  $\text{H}_2\text{SO}_4$  to obtain

a mixture containing slightly more than 82.38% total  $\text{SO}_3$ . If the fuming acid contains 50% excess  $\text{SO}_3$ , about 100 g of fuming acid to 140 g of concentrated acid will be approximately the correct ratio. Determine the exact strength of the mixture and also of a reserve supply of concentrated acid as follows:

Weigh a quantity of the acid in a weighing bulb or pipet having a capillary tube at the lower end and a stopcock at the upper end and fitted with a Pt wire for suspending on the balance. Fill the bulb by slight suction and empty the lower end of the capillary by closing the stopcock simultaneously with the withdrawal of the capillary from the acid, wiping off first with a moist and then with a dry cloth. Allow the acid to flow down the sides of the neck of a volumetric flask into cold  $\text{H}_2\text{O}$ . (If a flask approximately 100 times the volume of the weighing pipet is used, the resultant soln will be near 0.5 N.) Wash all traces of acid into the flask, taking precautions to prevent loss of  $\text{SO}_3$  fumes. Make to volume and titrate from a buret against standard alkali, using the indicator with which the alkali was standardized. Calculate the  $\text{SO}_3$  content of both acids and add sufficient concentrated acid to the fuming mixture to bring it to 82.38%. After mixing, determine the strength of this fuming mixture as before. The  $\text{SO}_3$  content of this acid must not vary more than +0.05% or -0.08% from 82.38%. The acid must be carefully protected against absorption of moisture from the air.

## 19

## DETERMINATION

Place 20 cc of the 38 N  $\text{H}_2\text{SO}_4$  in a graduated narrow-necked Babcock flask, stopper, and place in ice  $\text{H}_2\text{O}$  to cool. Add slowly from a pipet, 5 cc of the turpentine, gently shaking or rotating the flask and keeping the temp. at about 60-65° by continued immersion in ice  $\text{H}_2\text{O}$ . When the mixture no longer develops heat on shaking, agitate thoroly by vigorously shaking for about  $\frac{1}{2}$  min. Place the flask in a water bath and heat at 60-65° for 10 min., keeping the contents of the flask thoroly mixed by shaking vigorously not less than 6 times during the heating period. (CAUTION: If the shaking is too vigorous at first, there is danger of the escaping  $\text{SO}_3$ , forcing some of the mixture up over the mouth of the flask.) Cool to room temp. and fill the flask with  $\text{H}_2\text{SO}_4$  until the surface rises well into the graduated neck. Centrifuge for 5 min. at 1200 r.p.m., or for 10 min. at 900 r.p.m.; or allow to stand, lightly stoppered, for 12 hours. Read the volume of unpolymerized residue (middle of meniscus), calculate the percentage, record its consistency and color, and determine its refractive index at 20°.

By this method pure gum spirits of turpentine gives less than 2.0% residue, which has a straw or darker color, viscous consistency, and a refractive index of not less than 1.500. A limpid colorless residue with a refractive index of less than 1.500 indicates the presence of mineral oil. The unpolymerized residue from an adulterated oil represents from 60-80% of the total quantity of adulterant present.

## 20

*Sulfuric-Fuming Nitric Acid Method<sup>a</sup>—Official, First Action*

Place 50 cc of the turpentine in a 300 cc Kjeldahl or other long-necked flask, cool in ice  $\text{H}_2\text{O}$ , and add slowly with constant agitation 25 cc of  $\text{H}_2\text{SO}_4$ . Shake well to obtain complete reaction, keeping the flask cool. When the reaction is complete, cool thoroly and add 25 cc of  $\text{H}_2\text{O}$ . Distil the polymerized mixture in a current of steam, collecting 300 cc of total distillate. Separate the oil from the aqueous portions.

Place a volume of fuming  $\text{HNO}_3$  (sp. gr. 1.5) equal to 3 times the volume of the oil in a 200-250 cc separatory funnel and cool in ice  $\text{H}_2\text{O}$ . Add the oil cautiously

dropwise, shaking carefully and keeping the mixture cool. After all the oil has been added, allow the funnel to stand quietly, very lightly stoppered, about 30 seconds, until the oil has had a chance to come to the surface. Then draw off the acid and wash the remaining oil once with a little fuming  $\text{HNO}_3$ , once with  $\text{HNO}_3$ , and finally several times with  $\text{H}_2\text{O}$ . Measure the volume of the oil, record its consistency and color, and determine its refractive index at  $20^\circ$ . Pure gum spirits of turpentine gives less than 0.5% residue by this method.

## SELECTED REFERENCES

- <sup>1</sup> Ind. Eng. Chem. Anal. Ed., **6**, 122 (1934).
- <sup>2</sup> J. Assoc. Official Agr. Chem., **13**, 48 (1930).
- <sup>3</sup> U. S. Dept. Agr. Bur. Chem. Bull. **898**; U. S. Bur. Standards Circ. **86**.
- <sup>4</sup> Adopted by American Society for Testing Materials.
- <sup>5</sup> Chem. Ztg., **30**, 631 (1906); U. S. Dept. Agr. Bur. Chem. Circ. **85**; J. Assoc. Official Agr. Chem., **6**, 465 (1923).
- <sup>6</sup> J. Ind. Eng. Chem., **1**, 27 (1909); J. Assoc. Official Agr. Chem., **5**, 547 (1922); **9**, 55 (1926); **15**, 67 (1932).



## IX. PAINTS, VARNISHES, AND CONSTITUENT MATERIALS

### WHITE LINSEED OIL PAINTS<sup>1</sup>—OFFICIAL

1

#### REAGENTS

(a) *Extraction mixture*.—10 volumes ethyl ether, 6 volumes benzol, 4 volumes methyl alcohol, and 1 volume acetone.

(b) *Aqueous sodium hydroxide soln.*—Dissolve 100 g of NaOH and dilute to 300 cc.

(c) *Alcoholic sodium hydroxide soln.*—Dissolve NaOH in 95% ethyl alcohol in the proportion of about 22 g per 1000 cc. Let stand in a stoppered bottle. Decant the clear liquid into another bottle and keep well stoppered. This soln should be colorless or only slightly yellow when used; it will keep colorless longer if the alcohol is previously treated with NaOH (about 80 g to 1000 cc), kept at about 50° for 15 days, and then distilled.

(d) *Iodine soln.*—Dissolve I in glacial acetic acid that has a melting point of 14.7–15° and is free from reducing impurities, in such a proportion that 13 g of I will be present in 1000 cc of soln. The preparation of the I monochloride soln presents no great difficulty but it should be done with care and accuracy in order to obtain satisfactory results. There should be in the soln no sensible excess either of I or more particularly of Cl over that required to form the monochloride. This condition is most satisfactorily attained by dissolving in all of the acetic acid to be used the requisite quantity of I, using a gentle heat to assist the soln, if it is found necessary. Set aside a small portion of this soln, and pass dry Cl into the remainder until the halogen content of the soln is doubled. Ordinarily, it will be found that by passing the Cl into the main part of the soln until the characteristic color of free I has just been discharged, there will be a slight excess of Cl, which is corrected by the addition of the requisite amount of the unchlorinated portion until all free Cl has been destroyed. A slight excess of I does little or no harm, but excess of Cl must be avoided.

(e) *Standard sodium thiosulfate soln.*—Prepare and standardize as directed under XXXI, 18 (b).

(f) *Starch soln.*—Stir up 2–3 g of potato starch or 5 g of soluble starch with 100 cc of 1% salicylic acid soln, add 300–400 cc of boiling H<sub>2</sub>O, and boil the mixture until the starch is practically dissolved, then dilute to 1 liter.

(g) *Potassium iodide soln.*—Dissolve 150 g of KI free from iodate in H<sub>2</sub>O and dilute to 1000 cc.

(h) *Acid ammonium acetate soln.*—Mix 150 cc of 80% acetic acid, 100 cc of H<sub>2</sub>O, and 95 cc of NH<sub>4</sub>OH (sp. gr. 0.90).

(i) *Ammonium polysulfide.*—Pass H<sub>2</sub>S gas into 200 cc of NH<sub>4</sub>OH in a bottle immersed in running H<sub>2</sub>O or in iced H<sub>2</sub>O until the gas is no longer absorbed; then add 200 cc of NH<sub>4</sub>OH and dilute with H<sub>2</sub>O to 1000 cc. Digest this soln with 25 g of flowers of S for several hours and filter.

(j) *“Lead acid.”*—Mix 300 cc of H<sub>2</sub>SO<sub>4</sub> and 1800 cc of H<sub>2</sub>O. Dissolve 1 g of c. p. Pb acetate in 300 cc of H<sub>2</sub>O and add this to the hot soln, stirring meanwhile. Let stand at least 24 hours and siphon thru a thick asbestos filter.

(k) *Potassium permanganate soln.*—Dissolve 3.2 g of KMnO<sub>4</sub> in 1 liter of H<sub>2</sub>O.

<sup>1</sup> Methods (D 215–29) of the American Society for Testing Materials and adopted tentatively at the 1930 meeting of the A. S. T. M. These methods have been edited to conform in part to the style of this publication, but otherwise they are as published in the 1929 Supplement to Book of A. S. T. M. Standards. Under the standardization procedure of the A. S. T. M., these methods are under the jurisdiction of the A. S. T. M. Committee D-1 on Preservative Coatings for Structural Materials.

let stand 8-14 days, siphon off the clear soln (or filter thru an asbestos filter), and standardize as follows: In a 400 cc beaker dissolve 0.25-0.30 g (accurately weighed) of Bureau of Standards' Na oxalate in 250 cc of hot  $\text{H}_2\text{O}$  (80-90°) and add 15 cc of  $\text{H}_2\text{SO}_4$  (1+1). Titrate at once with the  $\text{KMnO}_4$  soln, stirring vigorously and continuously. The  $\text{KMnO}_4$  must not be added more rapidly than 10-15 cc per min., and the last 0.5-1 cc must be added dropwise with particular care to allow each drop to be fully decolorized before the next is introduced. The temp. of the soln should not be below 60° by the time the end point is reached. (Too rapid cooling may be prevented by allowing the beaker to stand on a small asbestos-covered hot plate during the titration. The use of a small thermometer as a stirring rod is most convenient.) The weight of Na oxalate used multiplied by 0.833 gives its Fe equivalent. Keep the  $\text{KMnO}_4$  soln in a glass-stoppered bottle painted black to keep out light.

The Fe value of the  $\text{KMnO}_4$  multiplied by 1.076 theoretically equals its Sb equivalent. However, for use in determining Sb, the  $\text{KMnO}_4$  is best standardized as follows: To 0.25 g of pure metallic Sb in a 500 cc Pyrex Erlenmeyer flask, add 12-15 cc of  $\text{H}_2\text{SO}_4$  and 10-12 g of  $\text{K}_2\text{SO}_4$ ; heat until all the Sb is dissolved, cool, dilute to 250 cc with  $\text{H}_2\text{O}$ , add 20 cc of  $\text{HCl}$ , cool to 10-15°, and titrate with the  $\text{KMnO}_4$  soln until a faint pink color is obtained. For special work, after digesting, dilute to 100 cc with  $\text{H}_2\text{O}$ , add 1-2 g of  $\text{Na}_2\text{SO}_3$ , and boil until all the  $\text{SO}_2$  is expelled. This is shown when no blue color is obtained with the starch-iodate paper; the volume will be reduced about one-half. Dilute to 250 cc with  $\text{H}_2\text{O}$ , add 20 cc of  $\text{HCl}$  (sp. gr. 1.19), and complete the titration as described.

(l) *Standard potassium ferrocyanide.*—Dissolve 22 g of the pure salt in  $\text{H}_2\text{O}$  and dilute to 1000 cc. To standardize, transfer about 0.2 g (accurately weighed) of pure metallic Zn or freshly ignited pure  $\text{ZnO}$  to a 400 cc beaker. Dissolve in 10 cc of  $\text{HCl}$  and 20 cc of  $\text{H}_2\text{O}$ . Drop in a small piece of litmus paper, add  $\text{NH}_4\text{OH}$  until slightly alkaline, then add  $\text{HCl}$  until just acid, and then 3 cc of  $\text{HCl}$ . Dilute to about 250 cc with hot  $\text{H}_2\text{O}$  and heat nearly to boiling. Run in the ferrocyanide soln slowly from a buret with constant stirring until a drop tested on a white porcelain plate with a drop of the uranyl indicator shows a brown tinge after standing 1 min. Run a blank with the same amounts of reagents and  $\text{H}_2\text{O}$  as in the standardization. Subtract the amount of ferrocyanide soln required for the blank from the amounts used in standardization and in titration of the sample. (The standardization must be made under the same conditions of temp., volume, and acidity as obtain when the sample is titrated.)

(m) *Uranyl indicator for zinc titration.*—A 5% soln of uranyl nitrate in  $\text{H}_2\text{O}$  or a 5% soln of uranyl acetate in  $\text{H}_2\text{O}$  made slightly acid with acetic acid.

(n) *Alkaline lead nitrate soln.*—Into 100 cc of  $\text{KOH}$  soln (56 g in 140 cc of  $\text{H}_2\text{O}$ ) pour a saturated soln of  $\text{Pb}(\text{NO}_3)_2$  (250 g in 500 cc of  $\text{H}_2\text{O}$ ) until the precipitate ceases to redissolve, stirring constantly while mixing. Let settle, filter thru asbestos, and dilute the clear filtrate with an equal volume of  $\text{H}_2\text{O}$ . (About 3 volumes of the  $\text{Pb}(\text{NO}_3)_2$  soln will be required for 1 of the  $\text{KOH}$ .)

(o) *Ammoniacal cadmium chloride or zinc sulfate soln.*—Dissolve 8 g of  $\text{CdCl}_2$  in 200 cc of  $\text{H}_2\text{O}$  and add 200 cc of  $\text{NH}_4\text{OH}$  (sp. gr. 0.90), or dissolve 50 g of  $\text{Zn}(\text{SO}_4)_2$  in 270 cc of  $\text{H}_2\text{O}$  and add 230 cc of  $\text{NH}_4\text{OH}$  (sp. gr. 0.90).

(p) *Standard potassium iodate soln.*—Dissolve 3.6 g of  $\text{KIO}_3$  and 39 g of  $\text{KI}$  in 1000 cc of  $\text{H}_2\text{O}$ . (For general work the theoretical sulfur titer of this soln should be used; for special work, the soln may be standardized against like material, such as a lithopone of known sulfide-S content.) The theoretical titer is based on standard  $\text{Na}_2\text{C}_2\text{O}_4$  and is obtained as follows: To 300 cc of  $\text{H}_2\text{O}$  in a 600 cc flask, preferably glass-stoppered, add 10 cc of  $\text{HCl}$  (sp. gr. 1.19) and 1 g of  $\text{KI}$ . Cool, and add 10 cc

of 0.1 *N*  $\text{KMnO}_4$  soln which has been standardized against  $\text{Na}_2\text{C}_2\text{O}_4$ . Swirl gently, stopper, and let stand for 5 min. Titrate the liberated *I* with standard  $\text{Na}_2\text{S}_2\text{O}_3$  soln until the color fades. Then add 10 cc of starch soln and continue the titration until the blue color is destroyed. Repeat the titration, substituting 10 cc of the iodate soln for the  $\text{KMnO}_4$  soln. Calculate the normality of the iodate soln.

(q) *Starch indicator for sulfur titration*.—(1) To 1000 cc of boiling  $\text{H}_2\text{O}$ , add a cold suspension of 6 g of starch in 100 cc of  $\text{H}_2\text{O}$  and boil vigorously for 5 min. Cool the soln, add 6 g of  $\text{ZnCl}_2$  dissolved in 50 cc of cold  $\text{H}_2\text{O}$ , thoroly mix, and set aside for 24 hours. Decant the clear supernatant liquid into a suitable container, add 3 g of  $\text{KI}$ , and mix thoroly. (2, optional) Prepare an emulsion of 6 g of soluble starch in 25 cc of  $\text{H}_2\text{O}$ , add a soln of 1 g of  $\text{NaOH}$  in 10 cc of  $\text{H}_2\text{O}$ , and stir the soln until it gelatinizes. Dilute to 1000 cc with  $\text{H}_2\text{O}$ , add 3 g of  $\text{KI}$ , and mix thoroly.

(r) *Starch-iodate paper*.—Impregnate filter paper with a soln obtained by heating 2 g of starch with 100 cc of  $\text{H}_2\text{O}$ , and, after soln, adding 0.2 g of  $\text{KIO}_3$  dissolved in 5 cc of  $\text{H}_2\text{O}$ .

(s) *Standard iodine soln for  $\text{SO}_2$* .—Place 15–20 g of pure  $\text{KI}$  in a liter volumetric flask, dissolve in as little  $\text{H}_2\text{O}$  as possible, and then add about 6.4 g of resublimed *I*. Shake until all the *I* is dissolved, dilute to the mark with  $\text{H}_2\text{O}$ , and mix. This soln is approximately 0.05 *N* and is standardized against 0.05 *N*  $\text{Na}_2\text{S}_2\text{O}_3$  to obtain its true normality.

(t) *Standard sodium thiosulfate soln for  $\text{SO}_2$* .—Prepare and standardize as described in (e), using 12.42 g of  $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ ; or the 0.1 *N* soln may be diluted with an equal volume of cold  $\text{CO}_2$ -free  $\text{H}_2\text{O}$ .

(u) *Ferric sulfate soln for titanium*.—Prepare a soln containing 2% of *Fe* as ferric sulfate as follows: Dissolve 20 g of pure *Fe* or plain *C* steel in a slight excess of  $\text{HCl}$ , oxidize with  $\text{HNO}_3$ , add about 80 cc of  $\text{H}_2\text{SO}_4$ , and heat until fumes of the latter are evolved. Cool, dilute with  $\text{H}_2\text{O}$  to 1000 cc, digest on a steam bath until sulfates are dissolved, and filter if necessary. To oxidize any ferrous *Fe* that may be present, add 0.1 *N*  $\text{KMnO}_4$  soln until a faint pink color persists for 5 min. Ferric ammonium sulfate may also be used.

(v) *Standard ferric sulfate soln for colorimetric determination of iron*.—Determine the strength of the ferric soln for the  $\text{TiO}_2$  determination in terms of *Fe* and dilute a portion of this soln until one is obtained of the strength 1 cc = 0.00001 g of *Fe*.

(w) *Potassium thiocyanate indicator*.—Prepare a 2% soln of the pure salt in  $\text{H}_2\text{O}$ .

## 2

## PRELIMINARY PROCEDURE

On receipt of a sample make a record of the label, noting especially the brand, the name of the manufacturer, and any statement as to composition and net contents. Weigh the unbroken package, open, note odor and condition of the contents, pour into a clean container, and mix thoroly by pouring from one container to the other, finally leaving the well-mixed sample in the second container, which shall be tightly closed. The well-mixed sample is used at once for the determinations described under "Methods." The original can and cover may be cleaned with gasoline, wiped dry, and then weighed. This weight subtracted from the original weight will give the net weight of the contents. If desired, the specific gravity of the paint may be determined and the weight per gallon calculated, and the volume of paint and the capacity of the container may be measured.

## 3

WATER<sup>1</sup>

Mix 100 g of the paint in a 250 cc flask with 75 cc of toluene. Place the flask in an

<sup>1</sup> A convenient apparatus for this determination is shown in Fig. 1 (b) of the Standard Method of Test for Water in Petroleum Products and Other Bituminous Materials (A.S.T.M. Designation: D 95-30, 1933 A.S.T.M. Standards, Pt. II, p. 891).

oil bath, connect with condenser, apply heat to the bath, and distil until about 50 cc of distillate has been collected in a graduate. The temp. in the flask should then be 105–110°. The number of cc of H<sub>2</sub>O collected under the toluene in the receiver is the percentage of H<sub>2</sub>O in the paint.

4

## VOLATILE THINNER

Weigh accurately 3–5 g of the paint into a tared flat-bottomed dish about 8 cm in diameter, spreading the paint over the bottom. Heat at 105–110° for 1 hour, cool, and weigh. Calculate the loss in weight as percentage of H<sub>2</sub>O and volatile thinner, subtract from this the percentage of H<sub>2</sub>O (3), and report the remainder as volatile thinner.

5

## NATURE OF THE THINNER

Transfer about 150 g of the paint to a 500 cc flask fitted with a 2-holed cork stopper carrying a spray trap connected with a vertical condenser. Thru the other hole in the stopper pass an influx tube for steam. (This tube should dip below the surface of the paint.) Heat the flask in an oil bath or an air bath at 100° and pass thru it a current of steam; with the steam still passing thru, raise the temperature of the bath to 130°. Catch the distillate in a small separatory funnel and continue distillation until 300 cc of H<sub>2</sub>O has been condensed. Portions of this H<sub>2</sub>O may be drawn from the cock of the separatory funnel from time to time, but care must be taken not to draw out any of the volatile thinner. Let the distillate stand until it separates into 2 layers, then draw off the H<sub>2</sub>O, and filter the volatile thinner thru a dry filter paper into a dry flask. If the thinner is apparently turpentine, examine the distillate as directed in Chap. VIII. If the thinner is a mixture of turpentine and mineral spirits, an approximate determination of the amount of turpentine may be made by the polymerization test specified under turpentine, VIII, 19. It should be noted that turpentine is slightly soluble in H<sub>2</sub>O (about 0.3–0.4 cc per 100 cc of H<sub>2</sub>O).

To test for benzol, add a few drops of the distillate to a small quantity of a mixture of HNO<sub>3</sub> and H<sub>2</sub>SO<sub>4</sub>, and heat cautiously. The characteristic odor of nitrobenzol will be noted if benzol is present.

If the thinner is apparently all mineral spirits, no further examination is necessary.

If the amount of turpentine in the thinner is so small that its presence is questioned, it may be detected by placing 2 drops of the distillate and 2–3 cc of CHCl<sub>3</sub> in a dry test tube and adding 1 drop of antimony pentachloride. A slow or slight change in color will indicate the absence of turpentine. A rapid change in color to a dark red or purple will indicate the possibility of turpentine. The I number for turpentine by the Wijs method under these conditions is approximately 340. An I number of 20 or over will give additional proof of the presence of turpentine and enable calculation of the approximate amount.

6

## PERCENTAGE OF PIGMENT

Strain a portion of the well-mixed sample thru a No. 80 sieve with an opening of .177 mm and a wire diameter of .119 mm to remove any skins and weigh accurately about 15 g of the strained paint in a weighed centrifuge tube. Add 20–30 cc of extraction mixture 1(a), mix thoroly with a glass rod, wash the rod with more of the extraction mixture, and add enough of the reagent to make a total of 60 cc in the tube. Place the tube in the container of a centrifuge, surround the tube with H<sub>2</sub>O, and counterbalance the container of the opposite arm with a similar tube, or a tube with H<sub>2</sub>O. Whirl at a moderate speed until well settled. Decant the clear supernatant liquid, repeating the extraction twice with 40 cc of extraction mixture and

once with 40 cc of ethyl ether. After drawing off the ether, set the tube in a beaker of  $H_2O$  at about  $80^\circ$  or on top of a warm oven for 10 min., then in an oven at  $105-110^\circ$  for 2 hours. Cool, weigh, and calculate the percentage of pigment. Grind the pigment to a fine powder, pass thru a No. 80 sieve to remove any skins, and preserve in a stoppered bottle.

## 7

## PERCENTAGE OF NON-VOLATILE VEHICLE

Add together the percentages of  $H_2O$ , of volatile thinner, and of pigment, and subtract the sum from 100. Report the remainder as non-volatile vehicle.

## TESTING NON-VOLATILE VEHICLE

## 8

## PREPARATION OF FATTY ACIDS

(a) To about 25 g of the paint in a porcelain casserole, add 15 cc of aqueous  $NaOH$ , 2(b), and 75 cc of ethyl alcohol, mix, and heat uncovered on a steam bath until all volatile thinner is driven off and saponification is complete. Add 100 cc of  $H_2O$ , boil, add  $H_2SO_4$  (sp. gr. 1.2) (8-10 cc in excess), boil, stir, and transfer to a separatory funnel to which some  $H_2O$  has been previously added. Draw off as much as possible of the acid aqueous layer and any insoluble or precipitated matter, wash once with  $H_2O$ , then add 50 cc of  $H_2O$  and 50 cc of ethyl ether. Shake very gently with a whirling action to dissolve the fatty acids in the ether, but not so violently as to form an emulsion. Draw off the aqueous layer and wash the ether layer with one 15 cc portion of  $H_2O$  and then with 5 cc portions of  $H_2O$  until free from  $H_2SO_4$ . Then draw off the  $H_2O$  layer completely. Transfer the ether soln to a dry flask and add 25-50 g of anhydrous  $Na_2SO_4$ . Stopper the flask and let stand with occasional shaking at a temp. below  $25^\circ$  until the  $H_2O$  is completely removed from the ether soln, which will be shown by the soln becoming perfectly clear above the solid  $Na_2SO_4$ . Decant this clear soln, if necessary, thru a dry filter paper into a dry 100 cc Erlenmeyer flask. Pass a rapid current of dry air (pass thru a  $CaCl_2$  tower) into the mouth of the Erlenmeyer flask and heat to a temp. below  $75^\circ$  on a dry hot plate until the ether is entirely driven off. It is important to follow all the details, as ether generally contains alcohol, and after washing with  $H_2O$  always contains  $H_2O$ . It is difficult to remove  $H_2O$  and alcohol by evaporation from fatty acids, but the washing of the ether soln and subsequent drying with anhydrous  $Na_2SO_4$  removes both  $H_2O$  and alcohol. Ether, in the absence of  $H_2O$  and alcohol, is easily removed from fatty acids by gentle heat. If the pigment settles out rapidly in a sample of the paint on standing so that sufficient vehicle can be poured off, or if sufficient vehicle is obtained by centrifuging the paint, it will be advantageous to saponify this separated vehicle and liberate and prepare the fatty acids as described.

(b) Instead of procedure (a) the following may be used, especially with samples that give trouble by the former: To about 50 g of paint in a porcelain casserole, add 30 cc of aqueous 30%  $NaOH$  and 125 cc of ethyl alcohol, mix, and evaporate on the steam bath until residue is dry. Transfer to a 400 cc beaker and boil with 200 cc of  $H_2O$ , add  $H_2SO_4$  (sp. gr. 1.2) (25 cc in excess), boil, stir, filter thru large coarse paper, and drain. Scrape the mass into a flask, shake violently with ether, centrifuge, decant into a separatory funnel, and wash with small amounts of  $H_2O$  until free of  $H_2SO_4$ . Transfer the ether soln to a dry flask containing about 40 g of anhydrous  $Na_2SO_4$  and allow to stand until the ether layer is clear. Decant the clear soln thru filter paper into a dry 100 cc flask. Pass a rapid current of dry air into the mouth of the flask and heat to a temp. below  $75^\circ$  on a dry hot plate until the ether

is entirely removed. Keep these prepared fatty acids in a stoppered flask and examine at once.

The above methods of preparing the fatty acids directly from the material, rather than from the extracted vehicle, are based upon past experience in sometimes obtaining too low results by the latter method. Occasionally, however, trouble is experienced in saponifying the entire material, due to interference of pigment. In this case it is permissible to save the extracted vehicle (6), evaporate the organic solvents on a steam bath, and saponify and prepare the fatty acids in the usual manner from this extract. If the I number obtained in this manner passes a given specification, no further work is necessary; if the I number is low, it will be necessary to repeat the work directly on the entire material.

The fatty acids prepared as above should be kept in a stoppered flask and examined at once.

#### 9 TEST FOR MINERAL OIL AND OTHER UNSAPONIFIABLE MATTER

Place 10 drops of the fatty acids (8) in a 50 cc test tube, add 5 cc of alcoholic soda 1(c), boil vigorously for 5 min., add 40 cc of  $H_2O$ , and mix. A clear soln indicates that not more than traces of unsaponifiable matter are present.

#### 10 IODINE NUMBER OF FATTY ACIDS<sup>1</sup>

Place a small quantity of the fatty acids (8) in a small weighing buret or beaker. Weigh accurately. Transfer by dropping about 0.15 g (0.10–0.20 g) into a 500 cc bottle having a well-ground-glass stopper, or an Erlenmeyer flask having a specially flanged neck for the I test. Reweigh the buret or beaker and determine the amount of sample used. (If desired the sample may be weighed in a small wide-mouthed vial and the vial containing the weighed sample placed in the bottle or flask.) Add 10 cc of  $CHCl_3$ . Whirl the bottle or flask to dissolve the sample. Add 10 cc of  $CHCl_3$  to 2 empty bottles or flasks like that used for the sample. Add to each bottle or flask 25 cc of the Wijs soln, 1(d), and let stand with occasional shaking for 1 hour in a dark place at a temp. of from 21 to 23°. Add 10 cc of the 15% KI soln and 100 cc. of  $H_2O$ , and titrate with standard  $Na_2S_2O_3$  soln, 1(e), using starch as indicator. The titrations on the 2 blank tests should agree within 0.1 cc. From the difference between the average of the blank titrations and the titration on the sample and the I value of the thiosulfate soln, calculate the I number of the sample tested. (I number is given in centigrams of I to 1 g of sample.)

#### ROSIN

#### 11 Liebermann-Storch Test<sup>2</sup>

To about 1 g of the fatty acids add 15 cc of acetic anhydride and shake until soln is complete. Pour a few drops of this soln on a white porcelain plate (a crucible cover serves well) and add a drop of  $H_2SO_4$  (sp. gr. 1.53). A fugitive violet color indicates rosin.

#### 12 Halphen-Hicks Test<sup>3</sup>

Test the fatty acids with the Halphen-Hicks reagent as follows:

*Soln A.*—Dissolve 1 part by volume of phenol in 2 parts by volume of  $CCl_4$ .

*Soln B.*—Dissolve 1 part by volume of Br in 4 parts by volume of  $CCl_4$ .

<sup>1</sup> If appreciable amounts of rosin or of unsaponifiable matter are found to be absent in the vehicle of a paint, the I number of the fatty acids gives the best indication (tho not proof) of the presence of linseed oil. An I number of less than 175 (Wijs) for the fatty acids is an indication that the non-volatile vehicle was not pure linseed oil.

<sup>2</sup> Lewkowitch, *Chemical Technology and Analysis of Oils, Fats and Waxes*, Vol. 1, p. 623 (1921).

<sup>3</sup> *J. Ind. Eng. Chem.*, 3, 86 (1911).

Add 1–2 cc of Soln A to about 1 g of the fatty acids and pour this mixture into a cavity of an ordinary porcelain color-reaction plate until it just fills the depression. Immediately fill an adjacent cavity with Soln B. Cover the plate with an inverted watch-glass and note the color, if any, produced in the former soln by the action of the Br vapors from Soln B. A decided purple or deep indigo blue color is an indication of the presence of rosin.

#### PIGMENT

##### 13

##### *Qualitative and Quantitative Examination*

A complete qualitative analysis, following the well-established methods, should be made and the quantitative scheme modified as required. Add acetic acid slowly to the pigment until all carbonate is decomposed (noting whether any  $\text{H}_2\text{S}$  is evolved); then add a large excess of acid  $\text{NH}_4$  acetate soln, 1(h), boil, filter, and test the filtrate for metals other than Pb and Zn (especially Ca and Ba). The absence of Ca in this filtrate indicates that the extending pigments contain no  $\text{CaCO}_3$  or  $\text{CaSO}_4$ ; the absence of Ba indicates that the extending pigments contain no  $\text{BaCO}_3$ .<sup>1</sup> Wash the matter insoluble in acid  $\text{NH}_4$  acetate soln with another portion of this soln, and finally with hot  $\text{H}_2\text{O}$ . This insoluble matter is dried, ignited, and tested for siliceous matter,  $\text{BaSO}_4$ , and Ti compounds. To test for the Ti compounds, place a small amount of the insoluble matter, or of the original sample (about 0.5 g), in a 250 cc Pyrex glass beaker; add 20 cc of concentrated  $\text{H}_2\text{SO}_4$  and 7–8 g of  $(\text{NH}_4)_2\text{SO}_4$ . Mix well, and boil for a few min. A residue denotes the presence of  $\text{SiO}_2$  or siliceous matter. Cool the soln, dilute with 100 cc of  $\text{H}_2\text{O}$ , heat to boiling, settle, filter, and wash with hot 5%  $\text{H}_2\text{SO}_4$  until free from Ti. The residue may be tested for Pb, Ba, and  $\text{SiO}_2$ . Add  $\text{H}_2\text{O}_2$  to a small portion of the filtrate; a clear yellow-orange color indicates the presence of Ti. Boil another portion of the filtrate with metallic tin or Zn. A pale blue to violet coloration indicates Ti. Treat another portion (about 1 g) of the pigment with 20 cc of  $\text{HCl}$  (1+1) and note whether any  $\text{H}_2\text{S}$  is evolved; boil the soln for about 5 min., add about 25 cc of hot  $\text{H}_2\text{O}$ , filter, and wash with hot  $\text{H}_2\text{O}$ . Render a small portion of the filtrate alkaline with  $\text{NH}_4\text{OH}$ , acidify with  $\text{HCl}$ , and add a little  $\text{BaCl}_2$  soln; a white precipitate ( $\text{BaSO}_4$ ) indicates the presence of a soluble sulfate. To another portion of the filtrate add a little  $\text{H}_2\text{SO}_4$  (a white precipitate indicates the presence of Pb, soluble Ba or both (some  $\text{CaSO}_4$  may also separate); filter, wash to remove free acid, and treat the precipitate with a few drops of  $\text{KI}$  soln (the formation of yellow  $\text{PbI}_2$  indicates the presence of Pb). The white precipitate may also be treated with  $\text{H}_2\text{S}$  water; the formation of black  $\text{PbS}$  indicates the presence of lead. To another portion of the original filtrate add  $\text{NH}_4\text{OH}$  until alkaline, render slightly acid with acetic acid, heat to boiling, and add a little  $\text{K}_2\text{Cr}_2\text{O}_7$  soln; a yellow or orange-yellow precipitate indicates the presence of Pb, soluble Ba or both. To another portion of the original filtrate add a few drops of  $\text{K}_4\text{Fe}(\text{CN})_6$  soln. A white precipitate with a bluish tinge indicates the presence of Zn. Pass into the remaining portion of the original filtrate a current of  $\text{H}_2\text{S}$  for 5–10 min., add an equal volume of  $\text{H}_2\text{O}$ , and pass  $\text{H}_2\text{S}$  into the soln for about 5 min.; filter, wash with  $\text{H}_2\text{S}$  water; then digest the precipitate with  $\text{NH}_4$  polysulfide, filter, acidify the filtrate with  $\text{HCl}$ , and warm; the presence of Sb is indicated by the separation of an orange colored precipitate. The filtrate from the  $\text{H}_2\text{S}$  precipitate may be tested for Ba, Ca, and Mg in the usual manner.

<sup>1</sup> If the original sample contained  $\text{BaCO}_3$  and  $\text{PbSO}_4$ ,  $\text{CaSO}_4$ , or other soluble sulfate, the soluble Ba will form with the soluble sulfate a precipitate of  $\text{BaSO}_4$ , which will be determined as "insoluble matter." If the sample contained  $\text{SrSO}_4$  or  $\text{SrCO}_3$ , some  $\text{SrSO}_4$  may be counted as  $\text{BaSO}_4$ , some Sr will count as soluble Ba, and some may be counted as CaO. This element is not separated, as it probably will not be encountered, or will be present as an impurity in the Ba and Ca compounds.

14

## SPECIFIC GRAVITY

If the determination of sp. gr. of the pigment is required, determine according to the Standard Methods of Test for Specific Gravity of Pigments (Serial Designation: D 153-27) of the American Society for Testing Materials.<sup>1</sup>

## SINGLE PIGMENTS

15

## BASIC CARBONATE OF LEAD

(a) *Total Lead (Gravimetric)*.—Dissolve 1 g in 20 cc of  $\text{HNO}_3$  (1+1) in a covered beaker, heating till all  $\text{CO}_2$  is expelled; wash off cover, add 20 cc of  $\text{H}_2\text{SO}_4$  (1+1), evaporate to fumes of  $\text{SO}_3$ , cool, and add about 150 cc of  $\text{H}_2\text{O}$  and 150 cc of alcohol; let stand in cold  $\text{H}_2\text{O}$  1 hour, filter on a Gooch crucible, wash with 95% alcohol, dry at  $110^\circ$ , and weigh  $\text{PbSO}_4$ ; calculate to  $\text{PbO}$  or to basic carbonate.<sup>2</sup> Instead of determining the Pb as sulfate, the sample may be dissolved by boiling with acetic acid; then dilute to about 200 cc with  $\text{H}_2\text{O}$ , make alkaline with  $\text{NH}_4\text{OH}$ , then acid with acetic acid, heat to boiling, and add 10-15 cc of a 10% soln of  $\text{K}_2\text{Cr}_2\text{O}_7$ ; heat till the yellow precipitate assumes an orange color. Let settle and filter on a Gooch crucible, washing by decantation with hot  $\text{H}_2\text{O}$  till the washings are colorless, finally transferring all the precipitate. Wash with 95% alcohol and then with ether; dry at  $110^\circ$  and weigh  $\text{PbCrO}_4$ . (Any insoluble matter should be filtered out before precipitating the Pb.)

(b) *Total Lead (Volumetric)*.—Dissolve 0.5 g of sample in 10 cc of  $\text{HCl}$ , boil till soln is effected, cool, dilute to 40 cc, and neutralize with  $\text{NH}_4\text{OH}$ . Add acetic acid until distinctly acid, dilute to 200 cc with hot  $\text{H}_2\text{O}$ , boil, and titrate with  $\text{NH}_4$  molybdate as follows:

Dissolve 4.25 g of  $\text{NH}_4$  molybdate in  $\text{H}_2\text{O}$  and make up to 1 liter. To standardize this soln, dissolve about 0.2 g of pure Pb foil in  $\text{HNO}_3$  (pure  $\text{PbO}$  or  $\text{PbSO}_4$  may also be used), evaporate nearly to dryness, add 30 cc of  $\text{H}_2\text{O}$ , then 5 cc of  $\text{H}_2\text{SO}_4$  (sp. gr. 1.84), cool, and filter. Drop filter with  $\text{PbSO}_4$  into a flask, add 10 cc of  $\text{HCl}$ , boil till completely disintegrated, and add 15 cc of  $\text{HCl}$ , 25 cc of  $\text{H}_2\text{O}$ , and  $\text{NH}_4\text{OH}$  till alkaline. Acidify with acetic acid, dilute to 200 cc with hot  $\text{H}_2\text{O}$ , and boil. Titrate, using an outside indicator of 1 part of tannic acid in 300 parts of  $\text{H}_2\text{O}$ .

It should be noted that when Ca is present, it forms a more or less insoluble molybdate, and results are apt to be high. With samples containing less than 10% of Pb, the Pb should be precipitated as  $\text{PbSO}_4$ , filtered, redissolved, and titrated as in the process of standardizing.

(c) *Lead Carbonate and Lead Hydroxide*.—Determine  $\text{CO}_2$  by evolution with dilute  $\text{HCl}$ , absorbing in soda lime or  $\text{KOH}$  soln. Calculate  $\text{CO}_2$  to  $\text{PbCO}_3$ , subtract  $\text{PbO}$  equivalent from total  $\text{PbO}$ , and calculate residual  $\text{PbO}$  to  $\text{Pb(OH)}_2$ .

The following method of A. N. Finn (unpublished) gives total basicity of a pure white lead: Place 2 g of pigment in an evolution flask, add a little  $\text{CO}_2$  free  $\text{H}_2\text{O}$ , connect up to the separatory funnel and condenser (Knorr type), add thru the funnel, finally washing down, 100 cc of 0.25  $N$   $\text{HNO}_3$ , boil, and absorb the  $\text{CO}_2$  in soda-lime tube in the usual manner (having  $\text{H}_2\text{SO}_4$  and  $\text{CaCl}_2$  drying tubes in train), and weigh. To the soln in the evolution flask add about 20 cc of neutral  $\text{Na}_2\text{SO}_4$  soln and titrate with 0.25  $N$   $\text{NaOH}$  soln (carbonate-free), using phenolphthalein.  $\text{CO}_2$  is calculated to  $\text{PbCO}_3$ . The amount of 0.25  $N$  acid corresponding to the  $\text{CO}_2$  is calculated and deducted from the total amount of 0.25  $N$  acid neutralized by the sample and the difference is calculated to  $\text{Pb(OH)}_2$ .

<sup>1</sup> 1933 A.S.T.M. Standards, Part II, p. 568.

<sup>2</sup> This method of weighing lead sulfate is not accurate in the presence of calcium compounds.



## BASIC SULFATE OF LEAD

(a) *Qualitative Analysis*.—Test for matter insoluble in acid ammonium acetate soln, 1(h), for calcium, for carbonates, and for any other impurities suspected, by the regular methods of qualitative analysis.

(b) *Moisture*.—Place 1 g of the sample in a tared, wide-mouthed, short weighing tube provided with a glass stopper. Heat with stopper removed for 2 hours at a temp. between 105 and 110°. Insert stopper, cool, and weigh. Calculate loss in weight as moisture.

(c) *Insoluble Impurity and Total Lead*.—In a 250 cc beaker, moisten 1 g of the pigment with a few drops of alcohol; add 50 cc of acid ammonium acetate soln, 1(h). Heat to boiling and boil for 2 min. Decant thru a filter paper, leaving any undecomposed matter in the beaker. To the residue in the beaker, add 50 cc of the acid ammonium acetate soln, heat to boiling and boil for 2 min. Filter thru the same paper and wash with hot H<sub>2</sub>O. If an appreciable residue remains, ignite and weigh as insoluble impurity. Unite the acid ammonium acetate solns, heat to boiling, and add dropwise, while stirring, a slight excess (total of 10–15 cc) of a 10% soln of dichromate. Heat until the precipitate assumes an orange color, let settle, filter on a weighed Gooch crucible, wash by decantation with hot H<sub>2</sub>O until the washings are colorless, and finally transfer all the precipitate to the crucible. Then wash with 10 cc of 95% ethyl alcohol and finally with 10 cc of ethyl ether. Dry at 105–110°, cool, and weigh PbCrO<sub>4</sub>. Calculate to PbO by multiplying by the factor 0.69.

(d) *Zinc Oxide*.—Weigh accurately about 1 g of the pigment, transfer to a 400 cc beaker, add 30 cc of HCl (1+2), and boil for 2 or 3 min. Add 200 cc of H<sub>2</sub>O and a small piece of litmus paper. Add NH<sub>4</sub>OH until slightly alkaline, render just acid with HCl, then add 3 cc of HCl, heat nearly to boiling, and titrate with standard potassium ferrocyanide as in standardizing that soln, 1(i). Calculate total zinc as ZnO.

(e) *Lead Sulfate*.—Treat 0.5 g of the pigment in a 400 cc beaker with a few drops of alcohol, add 10 cc of bromine H<sub>2</sub>O, 10 cc of HCl (1+1) and 3 g of NH<sub>4</sub>Cl. Cover with a watch-glass and heat on a steam bath for 5 min. Add hot H<sub>2</sub>O to give a total volume of about 200 cc, boil for 5 min., filter to separate any insoluble matter (a pure pigment should be completely dissolved), and wash thoroly with hot H<sub>2</sub>O. (The insoluble matter may be ignited, weighed, and examined qualitatively.) Neutralize the clear soln (original soln or filtrate from insoluble matter) in a covered beaker with dry Na<sub>2</sub>CO<sub>3</sub>, add 1 g more of dry Na<sub>2</sub>CO<sub>3</sub>, and boil 10–15 min. Wash off cover, let settle, filter, and wash with hot H<sub>2</sub>O. Redissolve the precipitate in HCl (1+1), reprecipitate with Na<sub>2</sub>CO<sub>3</sub> as above, filter, and wash thoroly with hot H<sub>2</sub>O. Acidify the united filtrates with HCl, adding about 1 cc in excess. Boil to expel Br, and to the clear boiling soln add slowly, while stirring, 15 cc of 10% BaCl<sub>2</sub> soln. Let stand on the steam bath for about 1 hour, filter on a weighed Gooch crucible, wash thoroly with boiling H<sub>2</sub>O, dry, ignite, cool, and weigh as BaSO<sub>4</sub>. Calculate to PbSO<sub>4</sub>, using the factor 1.3.

(f) *Calculations*.—Calculate the percentage of PbSO<sub>4</sub> to PbO by multiplying by the factor 0.736 and subtract the result from the percentage of PbO found under (c), reporting the difference as PbO. Report ZnO found under (d) as percentage of ZnO. Report moisture and insoluble matter as such.

## ZINC OXIDE

(a) *Total Zinc*.—Dissolve 0.25–0.3 g in 10 cc of HCl and 20 cc of H<sub>2</sub>O; make alkaline with NH<sub>4</sub>OH, then acid with HCl; add 3 cc more of HCl, dilute to about

250 cc with  $H_2O$ , heat nearly to boiling, and titrate with standard  $K_4Fe(CN)_6$  soln as in standardizing that soln, 1(l). Mn, Fe, and Cu interfere. If they are present in the sample, remove as follows: Add to the cool HCl soln of Zn 35 cc of a prepared soln of  $NH_4OH$  and  $NH_4Cl$  (50 cc  $NH_4OH$ , 20 g  $NH_4Cl$  and 75 cc  $H_2O$ ). Boil the soln very gently for a minute or two. Add saturated Br water and continue the boiling for a short time. Filter the hot soln and wash precipitate 10 times with a nearly boiling  $NH_4Cl$  mixture (100 g  $NH_4Cl$  and 50 cc  $NH_4OH$  made up to 1 liter with  $H_2O$ ). Drop a small piece of litmus paper in the filtrate and cautiously neutralize with HCl, finally adding 3 cc in excess. Dilute, if necessary, to about 200 cc with hot  $H_2O$ , heat nearly to boiling, and add 50 cc of saturated  $H_2S$  water. The mixture is now ready for titration with the  $K_4Fe(CN)_6$  soln.

(b) *Total Soluble Sulfur*.<sup>1</sup>—Moisten a 10 g sample with  $H_2O$ , add a few drops of Br and then HCl, boil to expel Br, filter from any insoluble matter, and wash with hot  $H_2O$ . Make alkaline with  $NH_4OH$ , then just slightly acid with HCl, heat to boiling, and add about 15 cc of hot  $BaCl_2$  soln. Let stand several hours (overnight), filter on a weighed Gooch crucible, wash well with hot  $H_2O$ , dry, ignite for 5 min., cool, and weigh as  $BaSO_4$ . Calculate to S.

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## LITHOPONE

(Ponolith, Jersey Lily White, Becton White, Charlton White, Orr's White)

(a) *Insoluble and Total Zinc*.—Take 1 g of the sample in a 200 cc beaker, add 10 cc of HCl, mix, and add in small portions about 1 g of  $KClO_3$ , then heat on the steam bath until about half of the liquid is evaporated. Dilute with  $H_2O$ , add 5 cc of  $H_2SO_4$  (1+10); boil, let settle, filter, wash, ignite, cool, and weigh the insoluble which should be only  $BaSO_4$ ; make a qualitative examination for  $Al_2O_3$  and  $SiO_2$ . The insoluble should be examined under the microscope for the presence of natural crystalline barytes. Sample may also be examined direct. Make filtrate from the insoluble alkaline with  $NH_4OH$ , then acid with HCl; add 3 cc more of HCl, dilute to about 250 cc with  $H_2O$ , heat nearly to boiling, and titrate with  $K_4Fe(CN)_6$  soln as directed under 17. Calculate to Zn.

(b) *Zinc Oxide*.—Weigh accurately 1 g of the lithopone, transfer to a 250 cc beaker (moisten with a few drops of alcohol if an extracted pigment), add about 100 cc of 1–3% acetic acid, stir vigorously but *do not heat*, cover, and let stand for 18 hours, stirring once every 5 min. for the first 30 min. Filter, wash with 1 to 3% acetic acid followed by  $H_2O$  until the washings give no test for Zn with  $K_4Fe(CN)_6$  soln. Dilute the clear filtrate to about 200 cc with  $H_2O$ , add 30 cc of HCl (1+2), and a small piece of litmus paper; add  $NH_4OH$  until slightly alkaline, render just acid with HCl, then add 3 cc of HCl, heat nearly to boiling, and titrate with standard  $K_4Fe(CN)_6$  soln as described under 1(l). Calculate to ZnO. Calculate this result to Zn, subtract from total Zn, and calculate the difference to ZnS. (Any  $ZnCO_3$  or  $ZnSO_4$  is included in the ZnO.)

(c) *Zinc Sulfide*.<sup>2</sup>—Place 0.5 g of pigment in evolution flask with about 10 g of “feathered” or mossy Zn, add 50 cc of  $H_2O$ ; insert the stopper carrying a separatory funnel and an exit tube. Run in 50 cc of HCl from the funnel, having previously connected the exit tube to 2 absorption flasks in series; first flask contains 100 cc of alkaline  $Pb(NO_3)_2$  soln, second flask, 50 cc of same as a safety device. After all the acid has run into the evolution flask, heat slowly, finally boiling until the first appearance of steam in the first absorption flask; disconnect, let the PbS settle,

<sup>1</sup> Method of G. Rigg.

<sup>2</sup> Evolution method of W. G. Scott, *White Paints and Painting Material*, p. 257; see also Blair, *Chemical Analysis of Iron*.

filter, wash with cold  $H_2O$ , then with hot  $H_2O$  till neutral to litmus paper and washings give no test for Pb. The PbS precipitate is dissolved in hot, dilute  $HNO_3$ , evaporated to fumes with  $H_2SO_4$  and finally weighed as  $PbSO_4$ . Calculate PbS or  $PbSO_4$  to ZnS.

The alkaline Pb soln is made as follows: Into 100 cc of KOH soln (56 g in 140 cc of  $H_2O$ ) pour a saturated soln of  $Pb(NO_3)_2$  (250 g in 500 cc of  $H_2O$ ) until the precipitate ceases to redissolve, stirring constantly while mixing. About 3 volumes of the Pb soln will be required for 1 of the alkali.

Instead of absorbing the evolved  $H_2S$  in alkaline  $Pb(NO_3)_2$  soln a soln of 8 g of  $CdCl_2$  in 250 cc of  $H_2O$  and 150 cc of  $NH_4OH$  (sp. gr. 0.90) may be used. The CdS precipitate may be filtered on a weighed Gooch, washed with  $H_2O$  containing a little  $NH_4OH$ , dried at  $100^\circ$ , and weighed. Calculate to ZnS. It is better to filter the CdS on a small filter and wash as above, then place filter and precipitate in a beaker and dissolve in HCl and  $KClO_3$  (keeping at room temp. at first), filter out any paper pulp or insoluble matter; make filtrate alkaline with  $NH_4OH$ , then just acid with HCl, heat to boiling, and precipitate with  $BaCl_2$  in usual manner. Filter, wash, ignite, and weigh  $BaSO_4$ . Calculate to ZnS.

For very rapid work the contents of the absorption flask, after all  $H_2S$  has been absorbed, may be washed into a vessel with cold  $H_2O$  and diluted to about 1 liter, acidified with HCl, and titrated with standard I soln. Use starch indicator. (The precipitate should be completely dissolved.) Prepare the I soln by dissolving about 12.7 g of pure resublimed I and 18 g of KI in a little  $H_2O$  and diluting to 1 liter.

## 19

## TITANIUM PIGMENT

(a) *Titanium Oxide*.—Transfer 0.5 g of the dried sample to a 250 cc Pyrex beaker and add 20 cc of  $H_2SO_4$  and 7–8 g of  $(NH_4)_2SO_4$ . Mix well and heat on a hot plate until fumes of  $H_2SO_4$  are evolved, and then continue the heating over a strong flame until soln is complete (about 5 min. of boiling) or it is apparent that the residue is composed of  $SiO_2$  or siliceous matter. Caution should be observed in visually examining this hot soln. Cool the soln, dilute with 100 cc of  $H_2O$ , stir, heat carefully to boiling while stirring, let settle, filter thru paper, and transfer the entire precipitate to the paper. Wash the insoluble residue with cold 5% (by volume)  $H_2SO_4$  until Ti is removed.

Dilute the filtrate to 200 cc and add about 10 cc of  $NH_4OH$  (sp. gr. 0.90) to lower the acidity to approximately 5%  $H_2SO_4$  (by volume).

Wash out a Jones reductor<sup>1</sup> with dilute 5% by volume  $H_2SO_4$  and  $H_2O$ , leaving sufficient  $H_2O$  in the reductor to fill to the upper level of the Zn. (These washings should require not more than 1 or 2 drops of 0.1 N  $KMnO_4$  soln to obtain a pink color). Empty the receiver, and put in it 25 cc (measured in a graduate) of ferric sulfate soln, 1 (u). Reduce the prepared Ti soln as follows<sup>2</sup>:

(1) Run 50 cc of the 5%  $H_2SO_4$  soln thru the reductor at a speed of about 100 cc per min.

(2) Follow this with the Ti soln.

(3) Wash out with 100 cc of 5%  $H_2SO_4$ .

(4) Finally run thru about 100 cc of  $H_2O$ . See that the reductor is always filled with soln or  $H_2O$  to the upper level of the Zn. Gradually release the suction, thoroly wash the glass tube that was immersed in the ferric sulfate soln, remove the receiver, and titrate immediately with 0.1 N  $KMnO_4$  soln. 1 cc of 0.1 N  $KMnO_4$  =

<sup>1</sup> Directions for preparing a Jones reductor may be found in Blair, *The Chemical Analysis of Iron*, 8th Ed., pp. 88–89, or Freadwell-Hall, *Analytical Chemistry*, Vol. 2, 5th Ed.

<sup>2</sup> Lundell and Knowles, *J. Am. Chem. Soc.*, 45, 2620 (1923).

0.0048 g of Ti or 0.008 g of  $\text{TiO}_2$ . Run a blank determination, using the same reagents and washing the reductor as in the above determination. Subtract this permanganate reading from the original reading and calculate the final reading to  $\text{TiO}_2$ . This will include Fe, Cr, As, and any other substance which is reduced by Zn and acid. See calculations under (b) for reporting  $\text{TiO}_2$ .

(b) *Iron Oxide*.—Weigh 1 g of the sample and treat as directed in (a) as far as "dilute with 100 cc of  $\text{H}_2\text{O}$ ," 6th line; transfer without filtering to a graduated 200 cc flask, cool, fill to the mark with  $\text{H}_2\text{O}$ , mix, let settle, and determine Fe colorimetrically as follows: Filter thru a dry filter paper, discarding the first 20 cc; transfer 50 cc of the clear filtrate to a clean 100 cc Nessler tube or other comparator. Add a drop or two of 0.1 N  $\text{KMnO}_4$  soln to oxidize any ferrous Fe. The faint pink color should persist for at least 5 min. Add 10 cc of KCNS or  $(\text{NH}_4)\text{CNS}$  soln, 1(w), dilute with  $\text{H}_2\text{O}$  to 100 cc and mix thoroly. Compare the color immediately with a series of standards, prepared side by side with the sample, in similar tubes. Prepare the standards from the standard ferric sulfate soln, 1(v), so as to have a range of from 0.000005 g Fe to 0.00004 g Fe (0.5–4.0 cc). Transfer the desired volumes of the standard ferric sulfate soln to 100 cc Nessler tubes containing 50 cc each of an acid soln made up by dissolving 8 g of  $(\text{NH}_4)_2\text{SO}_4$  in  $\text{H}_2\text{O}$ , adding 20 cc of  $\text{H}_2\text{SO}_4$ , cooling, diluting with  $\text{H}_2\text{O}$  to 200 cc, and mixing. Add a drop of 0.1 N  $\text{KMnO}_4$  soln (or sufficient to yield a pink color that will persist for 5 min.), and then 10 cc of the thiocyanate soln. Finally dilute all standards with  $\text{H}_2\text{O}$  to 100 cc and mix each thoroly.

NOTE.—For a single sample it is more convenient to run the standard Fe soln from a buret into a Nessler tube, containing 50 cc of acid soln (made by dissolving 8 g of  $(\text{NH}_4)_2\text{SO}_4$  in  $\text{H}_2\text{O}$ , adding 20 cc of  $\text{H}_2\text{SO}_4$ , cooling and diluting with  $\text{H}_2\text{O}$  to 200 cc, and mixing), a drop of 0.1 N  $\text{KMnO}_4$  soln, 10 cc of the thiocyanate soln, and then dilute with distilled  $\text{H}_2\text{O}$  until the depth of the color produced after diluting 100 cc and mixing, exactly matches that of the sample. From the buret reading calculate the amount of Fe. When using standards, the color comparisons must be made immediately.

Calculate the total Fe found to  $\text{Fe}_2\text{O}_3$  and report as such. Calculate the  $\text{TiO}_2$  equivalent by multiplying the  $\text{Fe}_2\text{O}_3$  result by the factor 1.003 and subtract this figure from the total  $\text{TiO}_2$  as determined in (a) and report the remainder as  $\text{TiO}_2$ .

Report all results on the dry or moisture-free basis.

## MIXED OR COMPOSITE PIGMENTS

### 20

#### MOISTURE<sup>1</sup> (MATTER VOLATILE AT 105–110°)

Place 1–2 g of the sample in a wide-mouthed, short weighing tube provided with a glass stopper. Heat with the stopper removed for 2 hours at a temp. between 105 and 110°. Insert the stopper, cool, and weigh. Calculate the loss in weight as moisture (matter volatile at 105–110°).

### 21

#### LOSS ON IGNITION

Ignite 1 g of the pigment in a porcelain crucible over a Meker burner to constant weight.<sup>2</sup>

### 22

#### INSOLUBLE MATTER

Moisten 1 g of the sample with a few drops of alcohol, cover, add 40 cc of  $\text{HCl}$  (1 + 1), and boil gently for 5–10 min. Wash off cover, evaporate to dryness, and heat

<sup>1</sup> On an extracted and dried pigment, this determination is of little value. If the original paint contained gypsum, a part of the combined  $\text{H}_2\text{O}$  of the latter will be driven off in the drying of the extracted pigment and in the "moisture" determination.

<sup>2</sup> This determination may serve as a rough or approximate check in many cases on the  $\text{CO}_2$ ,  $\text{H}_2\text{O}$ , etc.

at about 150° for 30 min. to 1 hour to dehydrate the residue. Moisten the residue with 4 cc of HCl, allow to stand a few minutes, dilute with 100 cc of hot H<sub>2</sub>O, boil a few minutes, filter hot thru paper, and wash with hot H<sub>2</sub>O till washings give no test for Pb and Cl. Ignite the paper and residue in a Pt or porcelain crucible, cool, and weigh total insoluble matter.<sup>1</sup> The insoluble matter may be filtered off on a Gooch crucible, washed with hot H<sub>2</sub>O, dried at 105°, cooled and weighed; then ignited, cooled, and weighed, when it is desired to get the loss on ignition (combined H<sub>2</sub>O, organic matter, etc.), or when the insoluble matter is not to be further examined. If the sample contains titanium pigment, practically all the TiO<sub>2</sub> will be found in the insoluble matter along with BaSO<sub>4</sub> and siliceous matter. Should an examination of the insoluble matter be necessary, it is advisable to remove the TiO<sub>2</sub> before proceeding further. The TiO<sub>2</sub> may be removed (or determined on a separate portion) as directed under 19. After removing the TiO<sub>2</sub>, the residue containing siliceous matter and BaSO<sub>4</sub> may be ignited to remove the filter. To determine BaSO<sub>4</sub>, mix the ignited insoluble matter with about ten times its weight of anhydrous Na<sub>2</sub>CO<sub>3</sub> (grinding the mixture in an agate mortar if necessary) and fuse in a covered Pt crucible, heating about 1 hour. Cool and place the crucible and cover in a 200 cc glazed porcelain casserole (a casserole is preferable to a beaker as silica is dissolved from glass when in long contact with a strong Na<sub>2</sub>CO<sub>3</sub> soln). Add about 100 cc of H<sub>2</sub>O and heat until the mass is disintegrated. Filter on paper into a 300 cc glazed porcelain casserole (leaving crucible and cover in the original casserole) and wash the casserole and filter thoroly with a hot soln of Na<sub>2</sub>CO<sub>3</sub> (1%). Place the casserole containing the crucible and cover under the funnel, pierce the filter with a glass rod, and wash the residue into the original casserole by means of a jet of hot H<sub>2</sub>O. Wash the paper with hot HCl (1+1) and then with hot H<sub>2</sub>O. Remove the crucible and cover. Evaporate the HCl soln to dryness, and heat at about 150° for 30 min. to 1 hour; moisten the residue with about 10 cc of HCl, dilute with 100 cc of hot H<sub>2</sub>O, boil a few minutes, filter hot thru the paper and wash thoroly with hot H<sub>2</sub>O. Dilute the filtrate to a volume of 300 cc, bring to boiling and add, dropwise, 5 cc of H<sub>2</sub>SO<sub>4</sub> (1+4). Allow to stand in a warm place about an hour, filter on a weighed Gooch crucible, wash with hot H<sub>2</sub>O, ignite, cool, and weigh as BaSO<sub>4</sub>. Subtract the sum of the percentages of BaSO<sub>4</sub> and TiO<sub>2</sub> from the percentage of total insoluble matter and report the result as the percentage of insoluble siliceous matter.<sup>2</sup>

To determine silica, acidify the filtrate from the BaCO<sub>3</sub> filtration with HCl, boil to expel CO<sub>2</sub>, evaporate to dryness, bake to dehydrate the silica, moisten with HCl, dilute with 100 cc of hot H<sub>2</sub>O, boil, and filter thru the same paper as was used to recover silica from the BaCO<sub>3</sub> portion. Wash thoroly with hot H<sub>2</sub>O and proceed as in silicate analysis.

If it is desired to determine magnesium, combine this last filtrate with the filtrate from the final BaSO<sub>4</sub> separation and test for Al<sub>2</sub>O<sub>3</sub> and MgO in the usual way. To recover MgO that may have dissolved in the procedure for the elimination of the TiO<sub>2</sub>, make the filtrate containing the TiO<sub>2</sub> just alkaline with NH<sub>4</sub>OH, bring to boiling, filter, and wash. The filtrate may be tested for MgO. Any Al<sub>2</sub>O<sub>3</sub> present will be precipitated along with the TiO<sub>2</sub>. To recover this, ignite and weigh as TiO<sub>2</sub> and Al<sub>2</sub>O<sub>3</sub>. Deduct for TiO<sub>2</sub> present in the sample; the difference is Al<sub>2</sub>O<sub>3</sub>.

## 23

### TOTAL LEAD AND ANTIMONY

Unite the filtrate and washings (total volume 150-200 cc) from the total insoluble matter, pass H<sub>2</sub>S into the soln until it is saturated, add an equal volume of

<sup>1</sup> See Ref. 1, under 15.

<sup>2</sup> Any soluble Al<sub>2</sub>O<sub>3</sub> (Fe<sub>2</sub>O<sub>3</sub>) and in most cases MgO, and sometimes some CaO, come from the siliceous pigment used. MgO generally denotes the presence of asbestos.

H<sub>2</sub>O, and again saturate with H<sub>2</sub>S. Filter, wash with H<sub>2</sub>O containing a little H<sub>2</sub>S and dissolve in hot HNO<sub>3</sub> (1+3), washing the paper with hot H<sub>2</sub>O. Add 10–20 cc of H<sub>2</sub>SO<sub>4</sub> (1+1), evaporate until copious fumes of H<sub>2</sub>SO<sub>4</sub> are evolved, cool, and add about 75 cc of H<sub>2</sub>O, and then about 75 cc of 95% ethyl alcohol. Stir, let settle, filter on a Gooch crucible, wash with dilute alcohol, and dry in an oven at 105–110°; or, ignite gently in a radiator<sup>1</sup> or muffle, cool, and weigh as PbSO<sub>4</sub>. Calculate to PbO.<sup>2</sup>

If the pigment contains Sb, filter and wash the sulfide precipitate as above; then wash the precipitate with a fine jet of H<sub>2</sub>O from the paper into a porcelain dish or casserole, add 25 cc of NH<sub>4</sub> polysulfide, 1(i), cover the vessel, and warm the mixture at 40–60° for 10–15 min. with frequent stirring. Wash off cover, filter thru the paper used in the first case, and wash with 2–3% Na<sub>2</sub>S or (NH<sub>4</sub>)<sub>2</sub>S soln. Discard the filtrate. Dissolve the residue in hot dilute HNO<sub>3</sub> (1+3), and determine the lead as PbSO<sub>4</sub>, as described above. Or, the original sulfide precipitate may be discarded and the Pb determined on a separate portion of the pigment as follows: To 1 g of the sample in a covered beaker, add 40 cc of HCl (1+1) and boil gently for 5–10 min. Wash off cover and evaporate to dryness. To the residue add sufficient HCl to dissolve the PbSO<sub>4</sub> (with pigments containing considerable amounts of PbSO<sub>4</sub>, it may be necessary to add 15–20 cc of HCl), add about 50 cc of hot H<sub>2</sub>O, boil a few min., filter hot thru paper, and wash with hot H<sub>2</sub>O until washings give no test for Pb. (If the sample contains no insoluble matter filtration is omitted.) To the filtrate add 20 cc of H<sub>2</sub>SO<sub>4</sub> (sp. gr. 1.84) and evaporate until dense white fumes of H<sub>2</sub>SO<sub>4</sub> are copiously evolved. Allow to cool, but not below 60°, and then add slowly 50 cc of H<sub>2</sub>O while the soln is agitated. Heat to boiling for several min. in order to insure complete soln of Sb sulfate. Allow the PbSO<sub>4</sub> to settle out until the supernatant liquid is clear, not letting the temp. fall below 60°. If the liquid does not clear quickly heat it for a longer period. When clear, pour the soln thru a weighed porcelain Gooch crucible with asbestos mat, decanting the soln as completely as possible without allowing more than a very small amount of PbSO<sub>4</sub> to go over into the crucible. Now add 10 cc more of H<sub>2</sub>SO<sub>4</sub> (sp. gr. 1.84) to the PbSO<sub>4</sub> in the original beaker, and boil for several min. Cool, add slowly 30 cc of H<sub>2</sub>O, and again heat to boiling for a few minutes; allow the soln to cool to about 60° and completely transfer the PbSO<sub>4</sub> to the Gooch crucible. Wash with "lead acid," 1(j), to remove soluble sulfates and finally wash free of acid with dilute alcohol (equal parts of ethyl alcohol or denatured alcohol and H<sub>2</sub>O). Dry in an oven at 105–110°, or ignite gently in a radiator or muffle. Calculate to PbO, or determine as chromate as described below.

If soluble compounds of Ba or Ca are present, BaSO<sub>4</sub> and CaSO<sub>4</sub> will be included with the PbSO<sub>4</sub>. If soluble SiO<sub>2</sub> is present, it will also be included with the PbSO<sub>4</sub>. In such cases, the PbSO<sub>4</sub> precipitate, after being washed with dilute alcohol, may be dissolved in acid NH<sub>4</sub> acetate, 1(h), and the Pb determined as PbCrO<sub>4</sub>, as described below. For ordinary work, the amount of BaSO<sub>4</sub> dissolved by the acetate treatment may be disregarded.

If the pigment contains no soluble Sb, Ba, or Ca compounds, the Pb may be determined directly on the original pigment, as follows: To 1 g of the sample in a covered beaker, add 25 cc of HNO<sub>3</sub> (1+1), and boil gently a few min. Wash off cover, evaporate to dryness on a steam bath, moisten with HNO<sub>3</sub>, add hot H<sub>2</sub>O, and heat a few min. Filter, and wash with hot H<sub>2</sub>O until washings are Pb-free. Add

<sup>1</sup> U. S. Geological Survey Bull. 700 (1919), p. 33.

<sup>2</sup> It is not possible to determine the amount of basic lead carbonate and lead sulfate when carbonates or soluble sulfates of other metals, such as calcium, are present. Neither basic lead carbonate nor basic lead sulfate is a definite compound.

10–20 cc of  $\text{H}_2\text{SO}_4$  (1+1) to the clear soln, evaporate, and determine Pb as  $\text{PbSO}_4$ , as above described.

In the absence of soluble compounds of Sb, Fe, Al, and Ba, the following procedure may be used: Treat 1 g of the original pigment with 25 cc of  $\text{HNO}_3$  (1+1) and proceed as above. To the clear soln, diluted to 200 cc, add  $\text{NH}_4\text{OH}$  in slight excess, acidify with acetic acid, and add 4–6 cc more of this acid; heat to boiling and add 10–15 cc of a 10% soln of  $\text{K}_2\text{Cr}_2\text{O}_7$ . Heat until the yellow precipitate assumes an orange color and let settle. Filter on a weighed Gooch crucible and wash by decantation until the washings are colorless, finally transferring all the precipitate. Wash with 95% alcohol and then with ether; dry to constant weight at  $110^\circ$ , cool, and weigh  $\text{PbCrO}_4$ . Calculate to  $\text{PbO}$ .

#### ANTIMONY OXIDE

##### 24

##### *Method I. In the *ous* condition*

Transfer 0.3 g of a straight antimony oxide pigment, or 0.5 g of a mixed pigment, to a 500 cc Pyrex Erlenmeyer flask, and add 15 cc of  $\text{H}_2\text{O}$  and 25 cc of  $\text{HCl}$ . Cover with a watch-glass, warm on the steam bath 10–15 min. to dissolve the antimony oxide, wash off cover, and add 250 cc of  $\text{H}_2\text{O}$  and 15 cc of  $\text{H}_2\text{SO}_4$ . Boil 2 min., cool to  $10$ – $15^\circ$ , and titrate to a faint pink tint with 0.1 *N*  $\text{KMnO}_4$  soln, 1(k). Calculate to  $\text{Sb}_2\text{O}_3$ .

##### 25

##### *Method II. In the *ous* and *ic* condition*

Transfer 0.3 g of a straight  $\text{Sb}_2\text{O}_3$  pigment, or 0.5 g of a mixed pigment, to a 500 cc Pyrex Erlenmeyer flask, add 15 cc of  $\text{H}_2\text{SO}_4$  (sp. gr. 1.84), 10 g of  $\text{K}_2\text{SO}_4$ , and a 9 cm filter paper (to furnish C to act as a reducing agent). Place a funnel in the neck of the flask, and heat until the soln becomes colorless. Cool, wash off the funnel, dilute to 250 cc with  $\text{H}_2\text{O}$ , add 20 cc of  $\text{HCl}$ , and boil 2 min.; cool to  $10$ – $15^\circ$ , and titrate to a faint pink tint with 0.1 *N*  $\text{KMnO}_4$  soln.<sup>1</sup>

##### 26

##### *Method III. In the presence of appreciable amounts of iron*

Treat 1 g of the mixed pigment, or 0.3 g of a straight  $\text{Sb}_2\text{O}_3$  pigment, in a covered 250 cc beaker with 5 cc of  $\text{H}_2\text{O}$  and 20 cc of  $\text{HCl}$  (sp. gr. 1.19); heat on the steam bath for 15 min., cool, wash off cover, add 3 g of tartaric acid and 100 cc of hot  $\text{H}_2\text{O}$ , and digest a few minutes. Filter, catching the filtrate in a 500 cc Pyrex Erlenmeyer flask; wash thoroly with hot  $\text{H}_2\text{O}$ , dilute to 300 cc with hot  $\text{H}_2\text{O}$ , and pass in  $\text{H}_2\text{S}$  until the precipitation is complete. (If the sample contains no insoluble matter, dissolve directly in a 500 cc Pyrex Erlenmeyer flask, add tartaric acid, dilute, and pass in  $\text{H}_2\text{S}$ .) Filter, wash with  $\text{H}_2\text{O}$  containing  $\text{H}_2\text{S}$  until free from  $\text{HCl}$ , return paper and precipitate to the Erlenmeyer flask, add 15 cc of  $\text{H}_2\text{SO}_4$  (sp. gr. 1.84) and 10 g of  $\text{K}_2\text{SO}_4$ , place a funnel in the neck of the flask, and heat until the soln is colorless. Cool, wash off the funnel, dilute to about 250 cc with  $\text{H}_2\text{O}$ , add 20 cc of  $\text{HCl}$  (sp. gr. 1.19), boil for 2 or 3 min., cool to about  $10^\circ$ , and titrate to a faint pink tint with 0.1 *N*  $\text{KMnO}_4$  soln, 1(k). Calculate the total antimony to  $\text{Sb}_2\text{O}_3$ .<sup>2</sup>

<sup>1</sup> If the digestion with  $\text{H}_2\text{SO}_4$  and  $\text{K}_2\text{SO}_4$  (plus filter paper) is continued after the solution becomes colorless, some of the antimony may be oxidized from the *ous* to the *ic* condition. In such cases, cool, wash off the funnel, dilute to 100 cc with  $\text{H}_2\text{O}$ , add 1–2 g of  $\text{Na}_2\text{SO}_3$ , and boil until all the  $\text{SO}_3$  is expelled. This is shown when no blue color is obtained with starch-iodate paper, 1(r); the volume will be reduced about one-half. Dilute to 250 cc with  $\text{H}_2\text{O}$ , add 20 cc of  $\text{HCl}$  (sp. gr. 1.19), and boil 2 min.; cool to  $10$ – $15^\circ$ , and titrate to a faint pink tint with 0.1 *N*  $\text{KMnO}_4$  soln. Calculate total Sb to  $\text{Sb}_2\text{O}_3$ . Subtract the  $\text{Sb}_2\text{O}_3$  found by the procedure given in the first paragraph under 25 from the total  $\text{Sb}_2\text{O}_3$  and calculate the residual  $\text{Sb}_2\text{O}_3$  to  $\text{Sb}_2\text{O}_3$ .

<sup>2</sup> See Ref. 1 above, omitting the last two sentences.

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## SOLUBLE BARIUM

Boil the combined filtrate and washings, reduced in volume by evaporation if need be, from the PbS precipitate (Total Pb) to expel  $\text{H}_2\text{S}$ . Add a slight excess of  $\text{H}_2\text{SO}_4$  (1+4) over the amount required to precipitate the Ba, heat to boiling, let stand on a steam bath about 1 hour, filter on a weighed Gooch crucible, wash with hot  $\text{H}_2\text{O}$ , dry, ignite, cool, and weigh  $\text{BaSO}_4$ .<sup>1</sup> Calculate to BaO.

28

ALUMINA ( $\text{Fe}_2\text{O}_3$ ,  $\text{TiO}_2$ ,  $\text{P}_2\text{O}_5$ )

Boil the filtrate from the PbS to expel  $\text{H}_2\text{S}$ , add a few drops of  $\text{HNO}_3$ , and continue the boiling a few min. to oxidize any Fe that may be present. In case soluble Ba was present, use the filtrate from that determination. To the soln containing at least 5 g of  $\text{NH}_4\text{Cl}$  per 200 cc of soln, or an equivalent amount of HCl, add a few drops of methyl red (0.2% alcoholic soln) and heat just to boiling. Carefully add dilute  $\text{NH}_4\text{OH}$  dropwise until the color of the soln changes to a distinct yellow. Boil the soln for 1 to 2 min. and filter at once. Wash the precipitate thoroly with hot 2%  $\text{NH}_4\text{Cl}$  soln.<sup>2</sup> Ignite the precipitate, cool, and weigh as  $\text{Al}_2\text{O}_3$ .<sup>3</sup>

## TOTAL ZINC

29

## Method I

To the combined filtrate and washings from the alumina precipitate, add sufficient  $\text{NH}_4\text{Cl}$  to give 5 g per 100 cc of soln, and then add 1 g of  $\text{NH}_4$  acetate.<sup>4</sup>

Render slightly acid with acetic acid and pass in a current of  $\text{H}_2\text{S}$  to saturation. Allow the precipitate to settle completely, filter on paper, and wash with a 2% soln of acetic acid saturated with  $\text{H}_2\text{S}$ . Transfer the precipitate and filter to the vessel in which the precipitation was effected, add 30 cc of  $\text{H}_2\text{O}$  and 10 cc of HCl, and heat until all Zn is in soln; add 200 cc of  $\text{H}_2\text{O}$  and a small piece of litmus paper; add  $\text{NH}_4\text{OH}$  until slightly alkaline, render just acid with HCl, then add 3 cc of HCl, heat nearly to boiling, and titrate with standard  $\text{K}_4\text{Fe}(\text{CN})_6$  soln as in standardizing that soln, 1(l).

30

## Method II

Determine zinc directly on the original sample as follows.<sup>5</sup> Weigh accurately about 1 g (or an amount that will give a buret reading approximately equal to that obtained in the standardization) of the pigment, transfer to a 400 cc beaker, add 30 cc of HCl (1+2), boil a few min., and add 200 cc of  $\text{H}_2\text{O}$  and a small piece of litmus paper; add  $\text{NH}_4\text{OH}$  until slightly alkaline, render just acid with HCl, then add 3 cc of HCl, heat nearly to boiling, and titrate with standard  $\text{K}_4\text{Fe}(\text{CN})_6$  soln as in standardizing that soln, 1(l).

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## Method III

When Fe is present, total Zn may be determined directly on the original sample as follows.<sup>6</sup> Weigh accurately about 1 g (or an amount that will give a buret reading approximately equal to that obtained in the standardization) of the pigment, trans-

<sup>1</sup> This will include any  $\text{BaSO}_4$  that may have been dissolved as such. The weighed precipitate should be tested for  $\text{CaSO}_4$ , and if present, it should be removed by treating with hot dilute HCl, filtering, washing, igniting, and again weighing. Also see Ref. 1 under 15.

<sup>2</sup> For very accurate work, or when the precipitate is large, the precipitate should be dissolved in HCl (1+1) and the precipitation repeated.

<sup>3</sup> This precipitate may also contain  $\text{Fe}_2\text{O}_3$ ,  $\text{TiO}_2$ , and  $\text{P}_2\text{O}_5$ .

<sup>4</sup> Gooch, *Representative Procedures in Quantitative Chemical Analysis*, 1st ed., p. 107.

<sup>5</sup> If the sample contains Sb, it should be precipitated by  $\text{H}_2\text{S}$  in the hot acid soln, filtered off, washed, and the filtrate neutralized, etc., for Zn. The  $\text{H}_2\text{S}$  precipitate may also contain PbS. If no sulfide separation is made, any Cd present will be counted as Zn.



fer to a 250 cc beaker, moisten with alcohol, add 30 cc of HCl (1+2), boil for 2 or 3 min., and add about 100 cc of H<sub>2</sub>O. Add about 2 g of NH<sub>4</sub>Cl, make slightly alkaline with NH<sub>4</sub>OH, heat to boiling, let settle on steam bath, filter into a 400 cc beaker, and wash the residue once with hot H<sub>2</sub>O. Remove the 400 cc beaker, pour dilute HCl on the residue, catching the filtrate therefrom in the 250 cc beaker, and wash a few times with hot H<sub>2</sub>O. Add to this filtrate 1 g of NH<sub>4</sub>Cl and make slightly alkaline with NH<sub>4</sub>OH, boil, let settle, filter on paper used for first filtration, and wash thoroly with hot H<sub>2</sub>O, catching the filtrate and washings in the 400 cc beaker containing the first filtrate. Add a small piece of litmus paper, acidify with HCl, add 3 cc of HCl, heat nearly to boiling, and titrate with standard K<sub>4</sub>Fe(CN)<sub>6</sub> as above.

## 32

## Method IV

With pigments containing ZnO and ZnS, the ZnO may be determined as follows: Weigh accurately 1 g of the pigment and proceed as directed under 18(b).

## 33

## SOLUBLE CALCIUM

Heat the united filtrate and washings, reduced in volume if need be, from the ZnS precipitate, to boiling, and add 1 cc of NH<sub>4</sub>OH and an excess of a hot saturated NH<sub>4</sub> oxalate soln. Continue the boiling until the precipitate becomes granular; let stand about 1 hour, filter, and wash with hot H<sub>2</sub>O. Ignite, cool, and weigh as CaO;<sup>1,2</sup> or place the beaker in which the precipitation was made under the funnel, pierce the apex of the filter with a stirring rod, and wash the precipitate into the beaker with hot H<sub>2</sub>O, pouring warm H<sub>2</sub>SO<sub>4</sub> (1+4) thru the paper and washing a few times. Add about 30 cc of H<sub>2</sub>SO<sub>4</sub> (1+4), dilute to about 250 cc, heat to 90°, and titrate at once with standard (0.1 N) KMnO<sub>4</sub> soln (the temp. of the soln should not be below 60° when the end point is reached. See Reagents). Calculate to CaO.<sup>3</sup> (The Fe value of KMnO<sub>4</sub> × 0.502 = CaO value.)

## 34

## SOLUBLE MAGNESIUM

Acidify the filtrate from the Ca precipitate with HCl, and add 10 cc of a saturated soln of NaNH<sub>4</sub>HPO<sub>4</sub> and NH<sub>4</sub>OH dropwise, with constant stirring. When the crystalline MgNH<sub>4</sub>PO<sub>4</sub> has formed, add 5 cc excess of NH<sub>4</sub>OH. Allow the soln to stand in a cool place for not less than 4 hours, preferably overnight;<sup>4</sup> filter, and wash with H<sub>2</sub>O containing 2.5% of NH<sub>3</sub>. Dissolve the precipitate in a small quantity of hot dilute HCl, dilute the soln to about 100 cc with H<sub>2</sub>O, add 1 cc of a saturated soln of NaNH<sub>4</sub>HPO<sub>4</sub> and NH<sub>4</sub>OH dropwise, with constant stirring, until the precipitate is again formed as described, and then add 5 cc excess of NH<sub>4</sub>OH. Let the precipitate stand in a cool place for not less than 2 hours, filter on a Gooch crucible, wash with H<sub>2</sub>O containing 2.5% of NH<sub>3</sub>, ignite, cool, and weigh as Mg<sub>2</sub>P<sub>2</sub>O<sub>7</sub>.<sup>5</sup> Calculate to MgO.

## 35

## CARBON DIOXIDE

Use 1-2 g of the pigment, depending upon the probable CO<sub>2</sub> content,<sup>6</sup> following

<sup>1</sup> Care must be exercised in this washing, as 1000 cc of boiling water will dissolve over 0.01 g of CaC<sub>2</sub>O<sub>4</sub>.

<sup>2</sup> For more accurate work, ignite the CaC<sub>2</sub>O<sub>4</sub> precipitate, cool, cautiously moisten with H<sub>2</sub>O, redissolve in HCl and dilute the soln to 100 cc. Add NH<sub>4</sub>OH in slight excess, boil the liquid, filter, and wash if a precipitate appears. Then reprecipitate the Ca with NH<sub>4</sub>OH and (NH<sub>4</sub>)<sub>2</sub>C<sub>2</sub>O<sub>4</sub> as above, filter, wash, ignite, cool, and weigh; or, titrate as described. See also Ref. 1 under 15.

<sup>3</sup> See Ref. 1 under 15.

<sup>4</sup> The less the amount of Mg present, the longer the precipitate must be allowed to settle.

<sup>5</sup> If the sample contains Mn, it will be caught in large part with the Mg<sub>2</sub>P<sub>2</sub>O<sub>7</sub>. If desired, Mn may be determined by dissolving the Mg<sub>2</sub>P<sub>2</sub>O<sub>7</sub> in HNO<sub>3</sub> and applying the bismuthate method.

<sup>6</sup> If the sample is high in sulfide, e.g., contains a high percentage of lithopone, grind 1-2 g of the pigment with dry K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>, transfer to the evolution flask, add 50 cc of H<sub>2</sub>O and run in H<sub>2</sub>SO<sub>4</sub> (1+1) from the separatory funnel. Or, place at the front of the purifying and drying train a tube containing acidified CuSO<sub>4</sub> soln, KMnO<sub>4</sub> soln, or CrO<sub>3</sub> soln.

either of the methods described under the Determination of Carbon Dioxide in the Standard Methods of Chemical Analysis of Limestone, Quicklime and Hydrated Lime (A.S.T.M. Designation: C 25-29) of the American Society for Testing Materials.<sup>1</sup>

## 36

TOTAL SOLUBLE SULFUR COMPOUNDS<sup>2</sup>

Treat 1 g of the pigment in a 400 cc beaker with 10 cc of H<sub>2</sub>O, 10 cc of HCl saturated with Br, and 5 g of NH<sub>4</sub>Cl. Digest (covered) on a steam bath for 5 min., dilute with hot H<sub>2</sub>O to about 200 cc, boil for 5 min., filter to separate any insoluble matter, and wash thoroly with hot H<sub>2</sub>O. Nearly neutralize the clear soln in a covered beaker with NaOH soln, complete the neutralization with dry Na<sub>2</sub>CO<sub>3</sub>, and add about 2 g more of this reagent. Boil 10-15 min., wash off cover, let settle, filter, and wash with hot H<sub>2</sub>O. Redissolve the precipitate in HCl (1+1), reprecipitate with Na<sub>2</sub>CO<sub>3</sub> as above, filter, and wash thoroly with hot H<sub>2</sub>O. Acidify the united filtrates with HCl, adding about 1 cc in excess. Boil to expel Br, and to the clear boiling soln add slowly with stirring an excess of a 10% BaCl<sub>2</sub> soln. Let stand on a steam bath for at least 1 hour, filter on a weighed Gooch crucible, wash thoroly with boiling H<sub>2</sub>O, dry, ignite at a dull red heat, cool, and weigh as BaSO<sub>4</sub>. This will include soluble sulfates, SO<sub>3</sub> formed from SO<sub>2</sub>, and the SO<sub>3</sub> that is formed from sulfide S.<sup>3</sup>

## 37

SOLUBLE SULFATE<sup>4</sup>

Treat 1 g of the pigment with 10 cc of H<sub>2</sub>O, 10 cc of HCl, and 5 g of NH<sub>4</sub>Cl. Boil until H<sub>2</sub>S is expelled, adding more HCl (1+1) if necessary; dilute with hot H<sub>2</sub>O to about 200 cc, boil for 5 min., filter to separate any insoluble matter, and wash thoroly with hot H<sub>2</sub>O. Nearly neutralize the clear soln with NaOH soln and make a double precipitation with Na<sub>2</sub>CO<sub>3</sub>, as in preceding method, finally weighing as BaSO<sub>4</sub>, as described above.<sup>3</sup>

## 38

SULFIDE SULFUR<sup>5</sup>

Place 0.5-1 g of the pigment in a flask with about 10 g of "feathered" or mossy Zn, and add 50 cc of H<sub>2</sub>O; insert a stopper carrying a separatory funnel and an exit tube. Run in 50 cc of HCl from the funnel, having previously connected the exit tube to 2 absorption flasks in series; the first flask contains 100 cc of alkaline Pb(NO<sub>3</sub>)<sub>2</sub> soln, 1(n), the second flask, 50 cc of the same soln as a safety device. After all the acid has run into the evolution flask, heat slowly, finally boiling until the first appearance of steam in the first absorption flask. Disconnect, let the PbS settle, filter, and wash with cold H<sub>2</sub>O, then with hot H<sub>2</sub>O till neutral to litmus paper and washings give no test for Pb. Dissolve the PbS precipitate in hot, dilute HNO<sub>3</sub>, and determine the Pb as PbSO<sub>4</sub>. Calculate to S. For very rapid work, the evolved H<sub>2</sub>S may be absorbed in an ammoniacal CdCl<sub>2</sub> or ZnSO<sub>4</sub> soln, 1(o), contained in 2 flasks connected in series, the contents of the absorption flasks washed into a vessel with cold H<sub>2</sub>O and diluted to about 1 liter, acidified with HCl, and titrated with standard KIO<sub>3</sub> soln, 1(p), using starch indicator, 1(q).

## 39

SULFUR DIOXIDE<sup>6</sup>

Transfer 10 g of the pigment to a suitable flask, insert a stopper fitted with a

<sup>1</sup> A.S.T.M. Standards, 1933, Pt. II, p. 55.

<sup>2</sup> See Ref. 1 under 15.

<sup>3</sup> See Ref. 3 under 28.

<sup>4</sup> See Ref. 1 under 15.

<sup>5</sup> Evolution Method of W. G. Scott, White Paints and Painting Materials, p. 257; see also Blair, The Chemical Analysis of Iron. The percentage of sulfide sulfur can be calculated from the percentages of total Zn and Zn soluble in 2-3% acetic acid, assuming the sulfide to be ZnS. See 32.

<sup>6</sup> This method is not applicable in the presence of sulfides decomposable under the conditions given.

separatory funnel and a spray trap delivery tube,<sup>1</sup> and attach the latter to a condenser. Place about 150 cc of HCl (1+3) in the funnel, the stopcock being closed,<sup>2</sup> and connect the other end of the condenser with a delivery tube which passes thru a 2-holed stopper and extends nearly to the bottom of an absorption flask; thru the other hole of the stopper connect a tube or flask to serve as a safety device. Place 25 cc of 0.05 *N* I soln, 1(s), in the absorption flask (dilute with H<sub>2</sub>O if necessary) and 20 cc of 10% KI soln in the safety tube; fit stopper in the absorption flask. Open the stopcock and allow the acid to slowly enter the flask. Before all the acid is admitted, air (washed with NaOH soln) is forced thru the top of the separatory funnel (about 2 bubbles per second in the KI soln). Boil the soln 3 min. with the air passing thru, then remove the source of heat and pass air thru for 30 min. Disconnect the absorption vessels, wash the KI soln into the I soln, and titrate at once with 0.05 *N* Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> soln, using starch indicator. Run a blank determination in exactly the same manner except for the omission of the pigment. Subtract this figure from the previous one and calculate the final result to SO<sub>2</sub> (1 cc of 0.05 *N* I = 0.0016 g of SO<sub>2</sub>).

## 40

## MATTER SOLUBLE IN WATER

Transfer 2.5 g of the pigment to a graduated 250 cc flask, add 100 cc of H<sub>2</sub>O and boil for 5 min. Cool to room temp., dilute to the mark with H<sub>2</sub>O, mix, and allow to settle. Filter the supernatant liquid thru a dry filter paper and discard the first 20 cc of the filtrate. Transfer 100 cc of the clear filtrate to a weighed dish, evaporate to dryness on a steam bath, dry for 1 hour in an oven at 105–110°, cool, and weigh. Calculate the percentage of water-soluble matter, the nature of which may be determined by further examination, as the percentages of SO<sub>3</sub> and CaO may be indicative.

## 41

## CALCULATIONS

The calculation of the component pigments of a mixed or combination pigment may be difficult. Depending upon the complexity of the mixed pigment, certain assumptions must be made as to the composition or formulas of component pigments and as to the manner in which the acidic and basic radicals are combined. Add any Al<sub>2</sub>O<sub>3</sub> (Fe<sub>2</sub>O<sub>3</sub>) found in the soluble portion to the siliceous matter and report the sum as "Insoluble siliceous matter," unless the soluble Al is high; in this case, an aluminate is probably present, and the Al<sub>2</sub>O<sub>3</sub> should be reported as Al<sub>2</sub>O<sub>3</sub>. If a small quantity of soluble Mg is found, it should also be added to the siliceous matter. If the soluble Mg is high, the presence of MgCO<sub>3</sub> is indicated, and the MgO is calculated to MgCO<sub>3</sub> as pointed out below. The insoluble siliceous matter reported should be based on the weight obtained on drying the total insoluble matter at 105° if the combined H<sub>2</sub>O contained therein is to be considered.

In the absence of ZnS or TiO<sub>2</sub>, report BaSO<sub>4</sub> as BaSO<sub>4</sub>. If ZnS is present, calculate the BaSO<sub>4</sub> equivalent by multiplying by 2.85; report sum of ZnS + BaSO<sub>4</sub> as "lithopone." If TiO<sub>2</sub> is present, calculate the BaSO<sub>4</sub> equivalent by multiplying by 3.17; report sum of TiO<sub>2</sub> + BaSO<sub>4</sub> as "titanium pigment." Report residual BaSO<sub>4</sub> as BaSO<sub>4</sub>. If TiO<sub>2</sub> is present and BaSO<sub>4</sub> is absent or is present in a smaller amount than would be indicated by the above factor, then report TiO<sub>2</sub> as TiO<sub>2</sub>, and BaSO<sub>4</sub> as BaSO<sub>4</sub>. If CaCO<sub>3</sub>, CaSO<sub>4</sub>, BaCO<sub>3</sub>, and MgCO<sub>3</sub> are absent, calculate CO<sub>2</sub> to basic carbonate white lead, (PbCO<sub>3</sub>)<sub>2</sub>. Pb(OH)<sub>2</sub>, and soluble SO<sub>3</sub> to PbSO<sub>4</sub>. Any excess of

<sup>1</sup> A Knorr CO<sub>2</sub> apparatus is convenient. In this case, the vertical condenser may be connected with an absorption tower containing the I soln, followed by the KI soln in a suitable tube.

<sup>2</sup> To minimize, if not eliminate, any possible oxidation by the air, add about 1 g (in one piece) of NaHCO<sub>3</sub> to the evolution flask, then add the acid directly to the flask, omitting the separatory funnel and the current of air. Boil the soln until about 50 cc of distillate has passed over.

Pb is calculated to PbO, added to the PbSO<sub>4</sub>, and the sum is reported as basic PbSO<sub>4</sub>; or, multiply the sum of PbSO<sub>4</sub>+PbO by 0.058 to obtain the ZnO; add this result to the PbSO<sub>4</sub>+PbO and report as basic sulfate white lead. (The ZnO factor is based on the assumption that the average composition of commercial basic sulfate white lead is: 78.5% PbSO<sub>4</sub>, 16.0% PbO, and 5.5% ZnO.) Lead oxide (PbO) should not be reported except in the presence of PbSO<sub>4</sub>, unless the entire analysis is reported in the elementary or oxide form.<sup>1</sup>

If the sample contains CO<sub>2</sub> but no soluble SO<sub>3</sub>, calculate total Pb to basic carbonate white lead, (PbCO<sub>3</sub>)<sub>2</sub>. Pb(OH)<sub>2</sub>; calculate residual CO<sub>2</sub> to CaCO<sub>3</sub>, then to BaCO<sub>3</sub> and MgCO<sub>3</sub> if soluble Ba and Mg should be present in sufficient amounts to indicate the presence of these carbonates. The CO<sub>2</sub> result will be an index of this. A small amount of residual CaO is probably from the siliceous matter and should be added to the insoluble siliceous matter.

A small amount of soluble Ba may be from the CaCO<sub>3</sub> used or may be due to the solubility of BaSO<sub>4</sub> if this compound is present in the original pigment. This Ba may be calculated to BaSO<sub>4</sub>, and added to the BaSO<sub>4</sub> found in the insoluble matter.

If the sample contains soluble SO<sub>3</sub> but no CO<sub>2</sub>, calculate CaO to CaSO<sub>4</sub> or CaSO<sub>4</sub>·2H<sub>2</sub>O; residual SO<sub>3</sub> to PbSO<sub>4</sub>; add residual PbO to PbSO<sub>4</sub> and report sum as basic PbSO<sub>4</sub>; or, multiply PbSO<sub>4</sub>+PbO by 0.058 and add the result to the PbSO<sub>4</sub>+PbO, and report the total as basic sulfate white lead.

If the sample contains CaCO<sub>3</sub> (MgCO<sub>3</sub>, BaCO<sub>3</sub>) and also basic sulfate white lead, or CaSO<sub>4</sub> and basic carbonate white lead, or a mixture of these, it is not possible to determine or calculate the amount of PbCO<sub>3</sub> or PbSO<sub>4</sub> with any degree of certainty.<sup>1</sup> The presence of appreciable amounts of CaO and SO<sub>3</sub> in the water-soluble matter indicates the probable presence of CaSO<sub>4</sub> in the original pigment. The following arbitrary calculations may be made: calculate water-soluble SO<sub>3</sub> to CaSO<sub>4</sub> or CaSO<sub>4</sub>·2H<sub>2</sub>O, subtract this SO<sub>3</sub> from total soluble SO<sub>3</sub> and calculate the remainder to PbSO<sub>4</sub>; calculate residual CaO to CaCO<sub>3</sub>, and then residual CO<sub>2</sub> to (PbCO<sub>3</sub>)<sub>2</sub>. Pb(OH)<sub>2</sub>. If there is an excess of CO<sub>2</sub>, calculate to MgCO<sub>3</sub> or BaCO<sub>3</sub>, if the amounts of soluble Mg and Ba indicate the probable presence of these carbonates. Add residual PbO to PbSO<sub>4</sub> and calculate, as above, to basic sulfate white lead. The procedure followed by the Federal Specifications Board should be noted.<sup>2</sup>

Report total Sb as Sb<sub>2</sub>O<sub>3</sub>.

Calculate sulfide S to ZnS, subtract the Zn equivalent to the S from the total Zn, then subtract the Zn required for the basic sulfate white lead, and report the remainder as ZnO.

Report moisture loss on ignition, SO<sub>2</sub>, and matter soluble in H<sub>2</sub>O directly.

## OLEO-RESINOUS VARNISHES<sup>3</sup>—OFFICIAL

### 42

#### PRELIMINARY PROCEDURE

Make all tests at room temp. between 21 and 32° (70 and 90°F.) and in diffused light (not in direct sunlight). After opening a can of varnish and using part of its contents, place the remainder immediately in air-tight containers which the varnish practically fills, leaving not more than 2% of air space.

<sup>1</sup> See Ref. 1 under 21 and Ref. 1 under 15.

<sup>2</sup> Federal Specifications Board Specification TT-P-36 for Paints: "The total lead dissolved in dilute acetic acid and hot ammonium acetate, weighed as lead sulfate, and this weight multiplied by the factor 0.883 shall be considered white lead. (It is not possible to determine the amount of PbCO<sub>3</sub> and PbSO<sub>4</sub> when carbonates or sulfates of other metals, such as Ca, are present. Also neither basic PbCO<sub>3</sub> nor basic PbSO<sub>4</sub> are definite compounds. The factor to convert PbSO<sub>4</sub> to (PbCO<sub>3</sub>)<sub>2</sub>Pb(OH)<sub>2</sub> is 0.854, to convert PbSO<sub>4</sub> to PbSO<sub>4</sub>·PbO is 0.868, and to convert PbSO<sub>4</sub> to (PbSO<sub>4</sub>)<sub>2</sub>·PbO is 0.913. The arbitrary factor used under this specification is the mean of the largest and smallest of these 3 factors.)"

<sup>3</sup> Standard Methods (D 154-28) of the American Society for Testing Materials edited to conform in part to the A.O.A.C. style. A.S.T.M. Standards, 1933, Pt. II, p. 698.

43

**APPEARANCE**

Pour some of the thoroly mixed sample into a clear glass bottle or test tube, 1.5–2.0 cm in diameter, to a depth of at least 2.5 cm. Then examine the varnish by transmitted light to see if it is clear and transparent.

44

**COLOR**

Prepare standard color solns by dissolving 1, 2, 3, 4, 5 and 6 g, respectively, of pure powdered  $K_2Cr_2O_7$  in 100 cc of  $H_2SO_4$ , applying gentle heat, if necessary, to effect soln of the  $K_2Cr_2O_7$ . (Since the  $K_2Cr_2O_7$ – $H_2SO_4$  solns must be freshly made for this color comparison, it is frequently more convenient to compare samples with a series of permanently sealed tubes of varnish which have been previously found to be lighter in color than the standard solns. Stabilized caramel solns or other permanent colored liquids may be used as secondary standards.)

Pour each of the standard color solns and a sample of the varnish to be tested into thin-walled glass tubes 1.5–2.0 cm in diameter to a depth of at least 2.5 cm. Make the color comparison by placing the tubes close together and looking thru them by transmitted light. If permanently sealed tubes of varnish are used for the color comparison and the sample of the varnish being tested is found to be darker than a standard tube of varnish, make final comparison with freshly prepared  $K_2Cr_2O_7$ – $H_2SO_4$  solns. State the color of the varnish in terms of the standard (calling the standards No. 1, No. 2, No. 3, etc.) which it is equal to or lighter than.

45

**NON-VOLATILE MATTER**

Pour a portion of the sample of the varnish into a stoppered bottle or weighing pipet and weigh. Transfer about 1.5 g of the sample to a weighed, flat-bottomed metal dish about 8 cm in diameter (a friction-top can plug is satisfactory). Reweigh the container and calculate by difference the exact weight of the portion of the sample transferred to the weighed dish. Heat the dish with its contents for 3 hours in an oven maintained at 105–110° and weigh after cooling.

Take the ratio of the weight of the residue to that of the sample, expressed in percentage, as the percentage of non-volatile matter in the varnish.

46

**ELASTICITY OR TOUGHNESS**

Proportionately reduce the elasticity or toughness of the varnish by the addition of a standard soln of kauri gum in pure spirits of turpentine, 47, or proportionately increase the elasticity or toughness of the varnish by means of addition of linseed oil, 48.

47

*Addition of Kauri Gum to Reduce Elasticity*

*Standard kauri gum soln.*—Arrange on a balance a distillation flask, water-cooled condenser, and a tared receiver. Place clear, bright, hard pieces of kauri gum broken to the size of a pea in the flask to about one-third its capacity. Carefully melt and distil the gum until 25% by weight is collected in the tared receiver, at which time of distillation the thermometer in the distillation flask, with the bulb at the level of the discharging end of the flask, should register about 316°. Pour the residue into a clean pan and when it is cold break it into small pieces. Place in a carefully tared beaker a quantity of the small broken pieces of kauri gum, together with twice its weight of freshly redistilled spirits of turpentine, using only that portion distilling between 153 and 170°, and dissolve by heating to a temp. of about 149°. When cooled, bring the mixture to correct weight by the addition of the quantity of redistilled spirits of turpentine necessary to replace the loss by evaporation during the dissolving of the gum.

Cut test panels from bright tin plate weighing not more than 25 g nor less than 19 g per sq dm (0.51–0.39 lb per sq ft). (It is important that the tin plate be within the limits prescribed. Commercial, No. 31-gage, bright tin plate should weigh about 0.44 lb per sq ft.) Use a panel about 7.5 by 13 cm and thoroly clean it with benzol immediately before using. (It is important to have the rags used in wiping the panels clean.)

Having carefully determined the non-volatile content of the varnish according to 45, take 100 parts of the varnish by weight, add a quantity of the standard kauri gum soln equivalent to 50% by weight of the non-volatile matter in the varnish, and mix thoroly. (A 20 g sample of varnish should be sufficient for each reduction.) If the non-volatile content of the varnish is 48.6%, add 4.86 g of standard kauri gum soln to the 20 g of varnish to obtain a 50% reduction. (The 50% standard kauri gum reduction is given to illustrate the method. Any other percentage of standard kauri gum reduction may be used, depending on the sample being tested.)

Flow the varnish upon one of the tin panels and stand the panel in a nearly vertical position at room temp. for nearly 1 hour. Then place the panel in a horizontal position in a properly ventilated oven and bake for 5 hours at 95–100°. Remove the panel from the oven and permit it to cool at room temperature (preferably 24°) for 15 minutes.

Place the panel with the varnished side uppermost over a 3 mm rod, held firmly by suitable supports, at a point equidistant from the top and bottom edges of the panel and bend double rapidly. The varnish should show no cracking whatsoever at the point of bending. For accurate results always bend the panel at 24°, as a lowering of the temp. will lower the percentage of reduction that the varnish will stand without cracking, while an increase in temp. will increase the percentage of reduction that the varnish will withstand.

Report varnishes which do not show cracks under this test as passing a 50% reduction, and report those that do crack as not passing a 50% reduction.

Re-test the varnishes which have not cracked, changing the amount of reduction to 60%; if they pass this percentage of reduction, test again with a 70% reduction. In a similar manner re-test varnishes which have cracked at 50%, using reductions of 40 and 30%. In this way determine the limits within 10% at which a varnish passes one percentage of reduction and does not pass the next. For example, varnishes may be reported as passing 40%, and breaking at 50%.

#### 48

##### *Addition of Linseed Oil to Increase Elasticity*

(Applicable to varnishes which are less elastic or less tough than zero kauri reduction.)

Follow the procedure described in 47, except to replace the 33 $\frac{1}{3}$ % soln of kauri gum in turpentine by a 66 $\frac{2}{3}$ % soln of heat-bodied linseed oil in order to proportionately increase the elasticity of the varnish under test.

*Standard bodied oil soln.*—Heat a high grade of alkali-refined linseed oil of an acid number less than 1.0 in an open kettle at a temp. of 300° ± 5° until the viscosity of the oil after cooling is between 6 and 10 poises at 25°. Standardize a quantity of the heat-bodied linseed oil by reducing it with one-half its weight of pure redistilled turpentine, using only that portion of turpentine distilling between 153 and 170°.

Conduct the addition of the standard bodied oil soln to the varnish, the flowing on, baking, and bending of the test panel exactly as in 47.

In reporting results, give the minimum percentage of the oil soln that must be added to the varnish, based on its non-volatile content, so that the final mixture

when flowed on a test panel and baked does not crack on the subsequent bending over a 3 mm rod.

## 49

FLASH POINT<sup>1</sup>

Use the "Tag" closed tester and make the test in a dim light so that the flash may be seen plainly. Surround the tester on three sides with an inclosure to keep away draughts. (A shield about 18 in. square and 2 ft. high, open in front, is satisfactory, but any safe precaution against all possible room draughts is acceptable. Tests made in a laboratory hood or near ventilators will give unreliable results.) See that the tester is firm and level. (For accuracy, the flash-point thermometers which are especially designed for the instrument should be used, as the position of the bulb of the thermometer in the sample cup is important.)

Put the water-bath thermometer in place, and put a receptacle under the overflow spout to catch the overflow. Fill the bath with H<sub>2</sub>O at such a temp. that, when testing is started, the temp. will be at least 20°F. below the probable flash point of the sample to be tested.

Put the sample cup in place in the water bath. Measure 50 cc of the sample to be tested in a pipet or a graduate, and place in the sample cup. Have the temp. of the sample at least 20°F. below its probable flash point when testing is started. Destroy any bubbles on the surface of the sample. Put on the cover with the flash-point thermometer in place and with a  $\frac{1}{8}$  in. rubber tube connecting the gas supply pipe to the gas connection on the cover. Light the pilot light on the cover and adjust the flame to the size of the small bead on the cover.

Light and place the heating lamp, filled with alcohol, in the base of the tester and see that it is centrally located. Adjust the flame of the alcohol lamp so that the temp. of the sample in the cup rises at the rate of about 1.8°F. per min., not faster than 2°F. nor slower than 1.6°F. per min.

Record the barometric pressure which, in the absence of a laboratory instrument, may be obtained from the nearest Weather Bureau Station. Also record the temp. of the sample at start.

When the temp. of the sample reaches 9°F. below the probable flash point of the sample, turn the knob on the cover so as to introduce the test flame into the cup, and turn it promptly back again. Do not let it snap back. The time consumed in turning the knob down and back should be about one full second, or the time required to pronounce distinctly the words "one-thousand-and-one." Record the time and the temp. of the sample when making the first introduction of the test flame.

Repeat the application of the test flame at every 1°F. rise in temp. of the sample until there is a flash within the cup. Do not be misled by an enlargement of the test flame or halo around it when it enters the cup, or by slight flickering of the flame; the true flash consumes the gas in the top of the cup and causes a very slight explosion. Record the time at which the flash point is reached and also record the flash point.

If the rise in temp. of the sample, from the time of making the first introduction of the test flame to the time at which the flash point is reached, was faster than 2°F. or slower than 1.6°F. per min., question the test and adjust the alcohol heating lamp to correct the rate of heating. It will be found that the wick of this lamp can be so accurately adjusted as to give a uniform rate of rise in temp. within the above limits.

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<sup>1</sup> Standard Method (D 56) of the A.S.T.M. edited to conform to the A.O.A.C. style. A.S.T.M. Standards, 1933, Pt. II, p. 663.

Having completed the preliminary test, remove the heating lamp, lift up the cover, and wipe off the thermometer bulb. Lift out the cup, and empty and carefully wipe it. Throw away all samples that have been used once in making a test.

Pour cold  $H_2O$  into the water bath, allowing it to overflow into a receptacle, until the temp. of the  $H_2O$  in the bath is lowered to  $15^{\circ}F.$  below the flash point of the sample, as shown by the previous test.

Place the cup in the bath and measure into it a 50 cc charge of fresh sample. Proceed to repeat the test as described above, introducing the test flame for the first time at a temp. of  $10^{\circ}F.$  below the flash point obtained on the previous test.

If two or more determinations agree within  $1^{\circ}F.$  consider the average of these results, corrected for barometric pressure, the flash point. If two determinations do not check within  $1^{\circ}F.$ , make a third determination and, if the maximum variation of the three tests is not greater than  $2^{\circ}F.$ , consider their average, after correcting for barometric pressure, the flash point.

Make correction for barometric pressure only in cases of dispute or when the barometer reading varies more than  $\frac{1}{2}$  in. (13 mm) from the standard pressure of 29.92 in. (760 mm). When the barometer reading is below this standard pressure, add to the thermometer reading  $1.6^{\circ}F.$  for each inch (25 mm) of barometric difference to obtain the true flash point. When the barometer reading is above the standard pressure, deduct  $1.6^{\circ}F.$  for each inch (25 mm) of barometric difference to obtain the true flash point.

## 50

## VISCOSITY

Determine viscosity by comparison at  $25^{\circ}$  with secondary standards (in bubble tubes) whose viscosity expressed in poises has been accurately determined at that temp.<sup>1</sup>

## 51

## WATER TEST

Pour the varnish on one of the tin panels described in 47, allow to drain in a nearly vertical position, and dry for 48 hours. Then place the panel in a beaker containing distilled  $H_2O$  at room temp. to a depth of about 7 cm, immersing the end of the panel which was uppermost during the drying, and allow to remain in the  $H_2O$  for 18 hours. Remove the panel from the  $H_2O$ , wipe carefully, and allow to dry out at room temp. Note the time required for whitening, if any, to disappear. Report the results as follows:

- (a) Not visibly affected.
- (b) Whitening disappears within 20 min.
- (c) Whitening does not disappear in 20 min., but does disappear within 2 hours.
- (d) Whitening does not disappear within 2 hours, but does disappear within 24 hours.
- (e) Whitening does not disappear within 24 hours.

Blooming, which sometimes occurs on immersion, is considered a degree of whitening.

RAW LINSEED OIL<sup>2</sup>—OFFICIAL, FIRST ACTION

## 52

## PREPARATION OF SAMPLE

Thoroughly agitate the sample before removing portions for analysis.

<sup>1</sup> Circ. 178, Scientific Section, Paint Manufacturers' Association of the United States.

<sup>2</sup> A.S.T.M. Standard Specifications for Raw Linseed Oil (D 234-28), A.S.T.M. Standards, 1933, Pt. II, pp. 644-649.



53

**SPECIFIC GRAVITY (APPARENT)**  
(15.5°/15.5°)

Use a pycnometer, accurately standardized and having a capacity of at least 25 cc, or any other equally accurate method, making the test at 15.5°, H<sub>2</sub>O being 1.000 at 15.5°.

**IODINE ABSORPTION NUMBER**

54

*Wijs Method*

Proceed as directed in 10, using from 0.09 to 0.15 g of oil.

**SAPONIFICATION NUMBER**

55

**REAGENT**

*Sulfuric acid soln.*—0.5 *N*. Add about 15 cc of H<sub>2</sub>SO<sub>4</sub> to distilled H<sub>2</sub>O, cool, and dilute to 1000 cc. Determine the exact strength by titrating against freshly standardized NaOH or by any other accurate method. Either adjust to exactly 0.5 *N* strength or leave as originally made, applying appropriate correction.

56

**DETERMINATION**

Weigh about 2 g of the oil in a 300 cc Erlenmeyer flask. Add 25 cc of alcoholic NaOH or KOH soln, 1(c). Put a condenser loop inside the neck of the flask and heat on the steam bath for 1 hour. Cool, add phenolphthalein as indicator, and titrate with 0.5 *N* H<sub>2</sub>SO<sub>4</sub>. Run two blanks with alcoholic NaOH soln, 1(c). These should check within 0.1 cc 0.5 *N* H<sub>2</sub>SO<sub>4</sub>. From the difference between the number of cc of 0.5 *N* H<sub>2</sub>SO<sub>4</sub> required for the blank and for the determination, calculate the saponification number (mg KOH required for 1 g of the oil).

57

**UNSAAPONIFIABLE MATTER**

Weigh 8–10 g of the oil and transfer to a 250 cc long-necked flask. Add 5 cc of a strong soln of NaOH (1+1) and 50 cc of 95% ethyl alcohol. Put a condenser loop inside the neck of the flask and boil for 2 hours. Occasionally agitate the flask to break up the liquid, but do not project the liquid onto the sides of the flask. At the end of 2 hours, remove the condenser and allow the liquid to boil down to about 25 cc.

Transfer the mixture to a 500 cc glass-stoppered separator, rinsing with H<sub>2</sub>O. Dilute with H<sub>2</sub>O to 250 cc, add 100 cc of redistilled ether, stopper, and shake for 1 min. Let stand until the two layers separate sharp and clear. Draw all but 1 or 2 drops of the aqueous layer into a second 500 cc separator and repeat the process, using 60 cc of ether. After thorough separation, draw off the aqueous layer into a 400 cc beaker, then the ether soln into the first separator, rinsing down with a little H<sub>2</sub>O. Return the aqueous soln to the second separator and shake out again with 60 cc of ether in a similar manner, finally drawing the aqueous soln into the beaker and rinsing the ether into the first separator. Shake the combined ether soln with the combined H<sub>2</sub>O rinsings and let the layers separate sharp and clear. Draw off the H<sub>2</sub>O and add it to the main aqueous soln. Shake the ether soln with two portions of H<sub>2</sub>O (about 25 cc each). Add these to the main H<sub>2</sub>O soln.

Swirl the separator so as to bring the last drops of H<sub>2</sub>O down to the stopcock and draw off until the ether soln just fills the bore of the stopcock. Wipe out the stem of the separator with a bit of cotton on a wire. Draw the ether soln (portionwise if necessary) into a 250 cc flask and distil off. While still hot drain the flask into a small weighed beaker, rinsing with a little ether. Evaporate this ether, cool the beaker,

and weigh. The unsaponifiable oil from adulterated drying oils may be volatile and as a consequence may evaporate on long heating. Therefore, heat the beaker on a warm plate, occasionally blowing out with a current of dry air. Discontinue heating as soon as the odor of ether is gone.

## ACID NUMBER

58

## REAGENT

*Standard sodium hydroxide soln.*—Prepare a stock concentrated soln by dissolving NaOH in H<sub>2</sub>O in the proportion of 200 g of NaOH to 200 cc of H<sub>2</sub>O. Allow this soln to cool and settle in a stoppered bottle for several days. Decant the clear liquid from the precipitate of Na<sub>2</sub>CO<sub>3</sub> into another clean bottle. Add clear Ba(OH)<sub>2</sub> soln until no further precipitate forms. Again allow to settle until clear. Draw off about 175 cc and dilute to 10 liters with freshly boiled, distilled H<sub>2</sub>O. Preserve in a stock bottle provided with a large guard tube filled with soda lime. Determine the exact strength by titrating against pure benzoic acid, using phenolphthalein as indicator. This soln will be approximately 0.25 N, but do not attempt to adjust it to any exact value. Determine its exact strength and make proper corrections in using it.

59

## DETERMINATION

Weigh 5–10 g of the oil and transfer to a 300 cc Erlenmeyer flask. Add 50 cc of a mixture of equal parts by volume of 95% ethyl alcohol and reagent benzol, previously titrated to a very faint pink with dilute alkali soln, using phenolphthalein as an indicator. Add phenolphthalein indicator and titrate at once to a faint permanent pink color with the NaOH soln. Calculate the acid number (mg KOH per g of oil).

## FOOTS (PER CENT)

60

## REAGENTS

(a) *Acid calcium chloride soln.*—Saturate with CaCl<sub>2</sub> a mixture of 90 parts of H<sub>2</sub>O and 10 parts of HCl.

(b) *Acetone.*—C. P. or A. S. T. M. D. 329.

61

## DETERMINATION

With all materials at a temp. of 20–27°, mix by shaking for exactly 1 min. in a graduated tube, 25 cc of the well-shaken sample of oil, 25 cc of acetone, and 10 cc of the acid CaCl<sub>2</sub> soln. Clamp the tube in a vertical position and allow the mixture to settle for 24 hours, keeping the temp. between 20 and 27°. Then determine the volume of the stratum lying between the clear CaCl<sub>2</sub> soln and the clear acetone and oil mixture to the nearest 0.1 cc or fraction thereof. This volume × 4 = the amount of foots present as a percentage by volume.

(The graduated tube may be a buret or a color comparison tube. It should have an internal diameter of 1.0–1.5 cm, and a capacity of not less than 70 cc. The graduations in 0.1 cc should extend at least 10–50 cc above the bottom of the tube.)

(a) *Heated oil test.*—Heat a portion of the oil to 65°, hold it within 2° of that temp. for 10 min., then cool it to room temp. (20–27°). Subject the sample promptly to the foots test as described above.

(b) *Chilled oil test.*—Heat a portion of the sample to 65°, and hold it within 2° of that temp. for 10 min. Then place it in a clean dry bottle, stopper tightly, and place in a cracked ice and H<sub>2</sub>O mixture (0°) for exactly 2 hours. At the end of this

time, place the bottle for exactly 30 min. in a  $\text{H}_2\text{O}$  bath at  $25^\circ$ , then subject promptly to the foots test as described above.

**62 LOSS ON HEATING AT  $105\text{--}110^\circ$**

Weigh 10 g of the oil in an accurately weighed 50 cc Erlenmeyer flask. Heat in an oven at a temp. of  $105\text{--}110^\circ$  for 30 min., while passing  $\text{CO}_2$  gas thru the oven. Then cool in a current of  $\text{CO}_2$  gas and weigh. Calculate the percentage loss.

**63 COLOR**

Prepare a fresh soln of 1 g of pure  $\text{K}_2\text{Cr}_2\text{O}_7$  in 100 cc of  $\text{H}_2\text{SO}_4$ . Place the oil and colored concentrated soln in separate thin-walled, clear-glass tubes of the same diameter (1–2 cm) to a depth of not less than 2.5 cm, and compare the depths of color by looking transversely thru the columns of liquid by transmitted light. State whether or not oil is darker than the colored soln.

**BOILED LINSEED OIL<sup>1</sup>—OFFICIAL FIRST ACTION**

**64 PREPARATION OF SAMPLE**

Proceed as directed under 52.

**65 SPECIFIC GRAVITY (APPARENT)  
( $15.5^\circ/15.5^\circ$ )**

Proceed as directed under 53.

**IODINE ABSORPTION NUMBER**

**66 *Wijs Method***

Proceed as directed under 54, using 0.09–0.15 g of oil.

**67 SAPONIFICATION NUMBER**

Proceed as directed under 55.

**68 UNSAPONIFIABLE MATTER (PER CENT)**

Proceed as directed under 57.

**69 ACID NUMBER**

Proceed as directed under 58.

**70 LOSS ON HEATING AT  $105\text{--}110^\circ$**

Proceed as directed under 62.

**71 TIME OF DRYING ON GLASS**

Flow the sample over a perfectly clean glass plate and place the plate in a vertical position in air that is at  $30^\circ \pm 2^\circ$  and of a humidity of  $32\% \pm 4\%$  saturation. After about 2 hours, test the film at intervals with the finger at points not less than 2.5 cm from the edges. The film shall be considered dry when it no longer adheres to the finger and does not rub up appreciably when the finger is lightly rubbed across the surface.

<sup>1</sup> A.S.T.M. Standard Specifications for Boiled Linseed Oil (D 260–33), A.S.T.M. Standards, 1933, Pt. II, pp. 638–643.

**72****ASH**

Weigh carefully in a weighed porcelain crucible or dish 10-25 cc of the sample and place on a stone slab on the floor of a hood. Ignite by playing the flame of a burner on the surface of the oil and allow to burn quietly until most of the oil is burned off; transfer to a muffle or over a flame and continue heating at a low temp. (not over a dull red) until all carbonaceous matter is consumed. Cool, weigh, and compute the percentage of ash.

**73****LEAD**

Dissolve the ash obtained as directed under **72** in  $\text{HNO}_3$  (1+10), to which a little  $\text{H}_2\text{O}_2$  has been added, and determine lead by the sulfate or any other equally accurate method.

**74****APPEARANCE**

Transfer a portion of the sample to a clear glass tube and note appearance. Report on clarity or presence of sediment.

## X. LEATHERS—TENTATIVE

### VEGETABLE TANNED LEATHER

#### 1 PREPARATION OF SAMPLE<sup>1</sup>

Reduce the leather by cutting, planing, sawing, shredding, grinding, or rasping to as fine a state of subdivision as is practicable. Avoid heating the sample during its preparation and especially do not use unsuitable grinding mills that cause heating. Mix thoroly and place in tightly covered containers.

#### MOISTURE<sup>2</sup>

##### 2 *Method I*

Place 5-10 g of the prepared sample, 1, in a tared, wide, shallow weighing bottle (or a similar dish, which can be covered tightly), and dry in an electric oven for 15 hours at 100-102°. Cover the weighing bottle, cool in a desiccator containing H<sub>2</sub>SO<sub>4</sub>, and weigh. The moisture in the leather as received may be determined by quickly cutting a representative portion of the sample into small pieces and drying as directed without further preparation.

##### *Method II—By Toluene Distillation*

#### 3 APPARATUS

- (a) 500 cc flask.—Erlenmeyer, or distilling flask of Pyrex or other resistant glass.
- (b) Receiving tube.—Graduated in tenths of a cc.
- (c) Liebig condenser.—Sealed-in, straight-tube, about 25 cm (10 in.) long, with delivery tube approximately 9.5 mm (0.375 in.) in diameter.

Assemble the apparatus as shown in XXVII, 3. Before each distillation clean the condenser and receiving tube with CrO<sub>3</sub>-H<sub>2</sub>SO<sub>4</sub> mixture; rinse thoroly with H<sub>2</sub>O, then with alcohol; and dry in an oven or with a current of air. Calibrate the receiving tube by distilling toluene containing known quantities of H<sub>2</sub>O. Read the volume of H<sub>2</sub>O to 0.01 cc.

#### 4 DETERMINATION

Weigh 20 g of the prepared sample, 1, and transfer to the distilling flask. Immediately add about 200 cc of dry toluene having a boiling point, under normal pressure, of 110-112°, and connect the flask with the receiving tube and condenser. Fill the receiving tube with toluene, pouring it thru the condenser. Heat the distilling flask gently and distil at the rate of about 4 drops per second for exactly 2 hours. At the end of 1, 1.25, 1.5, 1.75, and 2 hours' distillation, wash down the condenser by pouring toluene in at the top while brushing thoroly with a tight-haired, close-fitting tube brush that has been boiled previously in toluene. (A long handle may be made by fastening to the brush a piece of heavy Cu wire.) At the end of 2 hours, disconnect the receiving tube, dislodge any drops of H<sub>2</sub>O on the inside by rubbing with a piece of light Cu wire twisted at one end into a loop, and allow the tube to come to room temp. Read the volume of H<sub>2</sub>O to 0.01 cc and make such calibration correction as may be necessary. Assuming that 1 cc of H<sub>2</sub>O weighs 1 g, calculate the percentage of moisture.

#### 5 TOTAL ASH<sup>3</sup>

Incinerate slowly 5 g of the prepared sample, 1, at a dull red heat. If difficulty is experienced in burning off the C, leach the residue with hot H<sub>2</sub>O, filter on an ash-

less filter, dry and ignite the filter and residue, add the filtrate, evaporate to dryness, and ignite. Cool in a desiccator containing  $\text{H}_2\text{SO}_4$  and weigh.

The ash may be examined for acids and bases by any suitable method. Fe, Al, Mg,<sup>4</sup> Na, Ba, Ca, and Pb are the bases, and HCl and  $\text{H}_2\text{SO}_4$  are the acids which it may be necessary to determine.

## 6

INSOLUBLE ASH<sup>5</sup>

Quantitatively remove the leather remaining after the extraction of water-soluble material as directed under 9, dry at a temp. not exceeding 60°, weigh, and slowly incinerate a portion equal to exactly  $\frac{1}{4}$  of the total weight until all C is burned off. Cool in a desiccator containing  $\text{H}_2\text{SO}_4$  and weigh. Calculate the insoluble ash on the basis of the original leather represented.

## 7

PETROLEUM ETHER EXTRACT<sup>6</sup>

Place 5 g of the prepared leather, 1, in a fat-free paper thimble, cover with a layer of fat-free cotton, and extract in a Johnson or Soxhlet extractor for 8–10 hours with petroleum ether distilling between 50 and 80°. Heavily greased leathers (containing 15% or more fat) will require the maximum time. Remove the receiving flask, evaporate the petroleum ether on a steam bath, and dry the residue at 98–100° for periods of  $\frac{1}{2}$  hour each until a practically constant weight is obtained. Avoid prolonged continuous heating, resulting possibly in the partial volatilization or oxidation of the extract.

MINERAL ACIDITY<sup>6</sup>

## 8

*Modified Procter-Searle Method<sup>7</sup>*

Weigh 2 g of the prepared leather, 1, into a Pt dish; add 40 cc of a 0.1 N  $\text{Na}_2\text{CO}_3$  soln; mix thoroly, and evaporate to complete dryness on a steam bath. Place the residue in an electric muffle furnace at room temp., slowly raise the temp. of the muffle to  $600 \pm 10^\circ$  in 2 hours and maintain at this temp. for an additional hour. Remove the dish, allow to cool, and carefully moisten the residue with hot  $\text{H}_2\text{O}$ , adding about 25 cc, and break up lumps with a glass rod. Filter into a 300 cc flask thru an ashless paper and wash 4 or 5 times with hot  $\text{H}_2\text{O}$ . Return the filter paper and residue to its dish, dry, and incinerate in the muffle furnace at a dull red heat until all C is burned off. Cool, and add to the residue from a buret a quantity of 0.1 N  $\text{H}_2\text{SO}_4$  exactly equivalent to the  $\text{Na}_2\text{CO}_3$  originally added. Cover the dish and place on a steam bath for 30 min. Filter, if necessary, into the flask containing the first filtrate, washing the paper thoroly with hot  $\text{H}_2\text{O}$  until free from acid. Cool the soln and add 2 or 3 drops of methyl orange indicator (0.1 g per 100 cc  $\text{H}_2\text{O}$ ). If the soln is alkaline, no further titration is necessary, and the acidity is stated as "none." If the soln is acid, titrate to a distinct yellow color with the 0.1 N  $\text{Na}_2\text{CO}_3$  soln. Express the result as percentage of  $\text{H}_2\text{SO}_4$ . With each set of determinations run a blank thru the entire procedure, using the standard solns. If the blank is over 0.3 cc, repeat the determinations.

## 9

EXTRACTION OF WATER-SOLUBLE MATERIAL<sup>8</sup>

Weigh 30 g of the prepared leather, 1. (If the fat content of the sample, as determined by the petroleum ether extract, is more than 6%, extract the 30 g charge with petroleum ether, distilling between 50 and 80°, and allow the petroleum ether to evaporate spontaneously from the charge before proceeding with the extraction of water-soluble material.) Thoroly mix with the charge sufficient  $\text{H}_2\text{O}$  to soak and cover the leather. Transfer the leather and extract to a percolator that may be kept

at 50°. (The Reed-Churchill extractor<sup>9</sup> is especially convenient.) Extract at 50° by percolating with H<sub>2</sub>O at 50°, collecting 2 liters of percolate in 3 hours. Cool to room temp., dilute to exactly 2 liters, and mix thoroly.

To prevent fermentation add a few drops of toluene to the prepared extract and reserve it for the determination of glucose, soluble solids, and soluble non-tannins.

#### GLUCOSE<sup>10</sup>

10

##### REAGENTS

(a) *Di-potassium phosphate*.—Use only K<sub>2</sub>HPO<sub>4</sub> that is practically free from primary and tertiary salts, has been dried in thin layers at 98–100° for 16 hours, and kept in tightly stoppered bottles. A soln of the salt should have a pH value of about 9.0 and give a barely perceptible pink with phenolphthalein indicator.

(b) *Neutral lead acetate soln*.—Prepare as directed under XXXIV, 18(d).

(c) *Sozhlet's modification of Fehling's soln*.—Prepare as directed under XXXIV, 31.

(d) *Phenolphthalein soln*.—Dissolve 0.5 g of phenolphthalein in 100 cc of 95% alcohol.

(e) *Tartaric acid*.—Grind pure tartaric acid to a fine powder.

11

##### PREPARATION OF SOLUTION

To 200 cc of the prepared leather extract, 9, add by means of a pipet 25 cc of the neutral Pb acetate soln. Shake frequently for 5–10 min., then filter at once thru a dry, folded filter, returning the filtrate until it is clear. Keep the containers and funnel covered during these operations. Add to the filtrate 5.5 g of the dried K<sub>2</sub>HPO<sub>4</sub> (the quantity of K<sub>2</sub>HPO<sub>4</sub> must be not less than 4.5 nor more than 6.5 g). Shake frequently for 3–5 min., until all the phosphate has dissolved; then filter thru a dry, folded filter, returning the first runnings until the filtrate clears, and letting the funnel drain well. Pipet 150 cc of the filtrate into a 500 cc Erlenmeyer flask, and add by means of a pipet 7.5 cc of HCl. Also add about 25 mg of powdered stearic acid or 5–10 drops of kerosene to control frothing, and boil under a reflux condenser for exactly 2 hours. (If foaming occurs, turn off the flame, and when foaming subsides relight immediately. No further trouble should be experienced. After hydrolysis the acid soln may stand at laboratory temp. overnight without risk of loss of sugar.) Cool to 10–15°, add 2 drops of the phenolphthalein indicator, carefully neutralize with NaOH (1 + 1) added from a buret, and then add 0.5 cc in excess. *Without delay* transfer the soln to a 200 cc volumetric flask, complete to volume with H<sub>2</sub>O, and filter thru a double filter, returning the filtrate until it is clear. *During filtration keep the filtrate just acid by the addition from time to time of small quantities of pulverized pure tartaric acid. Immediately determine the dextrose in the soln.*

12

##### DETERMINATION

Pipet 50 cc of the prepared soln into a mixture of 25 cc of the Cu soln and 25 cc of the alkaline tartrate soln and proceed as directed under XXXIV, 37. Express the results as percentage of glucose on the leather basis, the 50 cc aliquot being equivalent to 0.5 g of leather.

13

##### SOLUBLE SOLIDS

If the H<sub>2</sub>O extract, prepared as directed under 9, is clear, proceed as directed under XI, 2; if it is cloudy, proceed as directed under XI, 5.

**14 SOLUBLE NONTANNINS**

Proceed as directed under **XI, 8**.

**15 SOLUBLE TANNIN**

The percentage of soluble tannin is the difference between **13** and **14**.

**16 NITROGEN**

Proceed as directed under **II, 20**.

**17 HIDE SUBSTANCE**

Multiply the percentage of N, obtained as directed under **16**, by the factor 5.62, to convert to the percentage of hide substance.

**18 COMBINED TANNIN**

Deduct the sum of the percentages of moisture, **2** or **3**; insoluble ash, **6**; petroleum ether extract, **7**; soluble solids, **13**; and hide substance, **17**, from 100. The remainder is the percentage of combined tannin.

## SELECTED REFERENCES

- <sup>1</sup> J. Am. Leather Chem. Assoc., **13**, 232 (1918); **14**, 321 (1919); **18**, 154 (1923); **23**, 412 (1928).  
<sup>2</sup> Ibid., **16**, 547 (1921); **17**, 262 (1922); **19**, 568 (1924); **20**, 334 (1925); **21**, 435 (1926); **22**, 265 (1927); J. Assoc. Official Agr. Chem., **10**, 31, 143 (1927).  
<sup>3</sup> J. Am. Leather Chem. Assoc., **13**, 7 (1918); **14**, 243 (1919); **15**, 130, 270 (1920).  
<sup>4</sup> Ibid., **16**, 595 (1921); **17**, 274, 592 (1922).  
<sup>5</sup> Ibid., **14**, 140, 499, 507 (1919); **16**, 458 (1921); **17**, 292, 540 (1922); J. Soc. Leather Trades Chem., **4**, 300 (1920).  
<sup>6</sup> Procter, Leather Industries Laboratory Book, 2nd ed., 1908, p. 369; J. Am. Leather Chem. Assoc., **14**, 330 (1919).  
<sup>7</sup> J. Am. Leather Chem. Assoc., **17**, 88 (1922); **18**, 430 (1923); **28**, 580 (1933); **29**, 259 (1934).  
<sup>8</sup> Ibid., **11**, 219 (1916); **13**, 142 (1918); **14**, 488 (1919); **15**, 581 (1920); **16**, 124, 264, 491 (1921); **17**, 220 (1922).  
<sup>9</sup> Ibid., **14**, 133 (1919).  
<sup>10</sup> Ibid., **7**, 645 (1912); **9**, 421 (1914); **15**, 411 (1920); **16**, 480 (1921); **17**, 284 (1922); **18**, 262, 459 (1923); **19**, 237, 339 (1924).



## XI. TANNING MATERIALS<sup>1</sup>—TENTATIVE

### EXTRACTS

1

#### PREPARATION OF SOLUTION

(a) *Solid and powdered extracts*.—Grind the sample, if necessary, as rapidly as possible in a porcelain mortar until all will pass a 10-mesh sieve of Cu or brass; mix thoroly and bottle. Weigh rapidly a quantity of sample containing 4 g of tannin (not less than 3.75 g nor more than 4.25 g).<sup>2</sup> Transfer to a beaker containing 100 cc of H<sub>2</sub>O at 85°, place on a steam bath, cover, and heat. Stir frequently until a homogeneous soln or suspension is obtained. Wash into a 1 liter flask with 800 cc of H<sub>2</sub>O at 85°. Allow to cool overnight at a temp. not below 19°, bring to 20° by placing the flask in H<sub>2</sub>O, the temp. of which is not below 19°, and dilute to 1 liter.

(b) *Liquid extracts*.—Let the sample come to room temp., mix thoroly, and weigh rapidly a charge yielding the same quantity of tannin as is specified under (a). Dissolve by washing into a liter flask with 900 cc of H<sub>2</sub>O at 85°. Allow to cool and dilute to 1 liter at 20°, as described under (a).

After the preparation of the soln proceed at once with the analysis.

2

#### TOTAL SOLIDS

Thoroly mix the prepared soln, 1, and pipet at once 100 cc into a weighed flat-bottomed glass dish, 2½–3 in. in diameter. (1) Evaporate and dry for 16 hours in a combined evaporator and dryer<sup>3</sup> at 98–100°, or (2) evaporate on a steam bath and then dry for 12 hours on the bottom of a water oven at 98–100°. Remove immediately to a desiccator containing H<sub>2</sub>SO<sub>4</sub> (place no more than 2 dishes in one desiccator) and weigh rapidly when cooled. Calculate the percentage of total solids.

#### SOLUBLE SOLIDS

3

#### REAGENT

*Kaolin*.—Should be neutral to phenolphthalein and should not yield more than 1 mg of soluble solids per 100 cc of filtrate of a 1% suspension in H<sub>2</sub>O after an hour's digestion at 20°.

4

#### PREPARATION OF FILTER

To about 75 cc of the prepared soln, 1, add 1 g of the kaolin. Stir, and pour immediately into a single, 15 cm No. 590, S. & S. or No. 1F Swedish filter.<sup>4</sup> (These papers must be pleated by hand as they are not available in folded form.) Return the filtrate to the paper when approximately 25 cc has run thru and repeat the operation for an hour, thus transferring all the kaolin to the paper. At the end of an hour discard the soln on the filter by siphoning it off, disturbing the kaolin as little as possible. An ordinary wash bottle serves well for this purpose.

5

#### DETERMINATION

Bring about 150 cc of the original prepared soln, 1, to exactly 20°. Fill the filter, prepared as directed under 4, with this soln and discard the filtrate until it runs thru clear. Keep the filter full, the temp. of the filtering soln at 20–25°, and the funnel and receiving vessel covered. Pipet at once 100 cc of the clear filtrate into a weighed dish, evaporate, and dry as directed under 2. Calculate the percentage of soluble solids.

6

#### INSOLUBLE SOLIDS

The percentage of insoluble solids is the difference between Total Solids (2) and Soluble Solids (5).

## NONTANNINS

7

## REAGENT

*Hide powder.*<sup>5</sup>—This should be of wooly texture, well delimed, and 10 g of the water-free powder should require 12–13 cc of 0.1 N NaOH to neutralize it.

Calculate the quantity of air-dried hide powder that will be required for the number of determinations to be made, on the basis of 12.5 g of H<sub>2</sub>O-free powder for each determination. Increase this calculated amount by 10 g of air-dried hide powder to provide a sufficient quantity for the determination of moisture in the wet chromed hide powder and also for a working leeway.

Digest the total quantity of air-dried hide powder with 10 times its weight of H<sub>2</sub>O until thoroly soaked. Then, for each g of the air-dried hide powder, so digested, add 1 cc of a 3% chrome alum soln, K<sub>2</sub>SO<sub>4</sub>Cr<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub>·24H<sub>2</sub>O, and either agitate frequently for several hours and let stand overnight, or agitate in some form of mechanical shaker for an hour<sup>6</sup>. Transfer to a strong linen filter and squeeze thoroly; using the linen filter as a bag, leave the hide powder in it and digest for 15 min. with a quantity of H<sub>2</sub>O equal to 15 times the weight of the air-dried hide powder used. Filter, and squeeze to approximately 73% of H<sub>2</sub>O, using a press if necessary. (Strong pressure is required to reduce the H<sub>2</sub>O content below 70%.) Repeat the digestion and filtration 3 times. The wet chromed hide powder, as finally prepared, should contain as nearly as possible 73% of H<sub>2</sub>O. The moisture content must be not less than 72% nor more than 74%.<sup>6</sup> Determine moisture in 20 g of the squeezed hide powder as directed under 2.

8

## DETERMINATION

Place 46 g of the prepared wet hide powder, 7, in a shaker bottle of suitable capacity; add 200 cc of the prepared tanning soln, 1, and shake immediately for 10 min. in a mechanical shaker. Squeeze at once thru linen; add 2 g of kaolin, 3, to the filtrate that contains the nontannins; stir; and filter thru a single, *folded* 18.5 cm filter paper (No. 1F Swedish preferred), refiltering until the filtrate is clear. Test the filtrate with gelatin-salt soln (1% gelatin and 10% salt), and if a precipitate forms, report the fact. Pipet 100 cc of the filtrate into a weighed dish and evaporate as directed under 2. Correct the weight of the nontannin residue for the dilution caused by the H<sub>2</sub>O retained in the wet hide powder. Calculate the percentage of nontannins.

9

## TANNIN

The percentage of tannin is the difference between Soluble Solids (5) and Nontannins (8).

SUGARS<sup>7</sup>

10

## PREPARATION OF SOLUTIONS

To 400 cc of prepared soln, 1, add 50 cc of Pb acetate soln, XXXIV, 18(d), shake well, and let stand for 5–10 min. Filter thru a folded filter (18.5 cm), returning the filtrate until it is clear. Let the filter drain for about 30 min. after all the soln has been poured. Remove the excess Pb from the filtrate with dried K<sub>2</sub>HPO<sub>4</sub>, X, 10(a), using the phosphate in the proportion of 5 g to 200 cc of the filtrate. (Measure the filtrate in a graduated cylinder. Usually 360–380 cc will be obtained, requiring 9–9.5 g of K<sub>2</sub>HPO<sub>4</sub>. Weigh the phosphate to within 0.1 g.) After adding the phosphate, shake well for 4 or 5 min. and filter thru a folded filter (18.5 cm).

## 11

## DETERMINATION

(a) *Reducing sugars*.—Place in a flask 100 cc of clarified deleadcd soln, 10, add 33.3 cc of  $H_2O$ , and if the reduction is not made at once, 8–10 drops of toluene; shake well and stopper with a plug of cotton. Keep in a cool place and make the reduction within 24 hours. When ready for reduction, filter if toluene has been added. Determine reducing sugars in duplicate 50 cc aliquots, as directed under XXXIV, 37. After correcting the weight of the  $Cu_2O$  for the blank of the Fehling's soln, find the equivalent mg of dextrose from XLII, 9. To express as percentage of dextrose, multiply the mg of dextrose by 3 and divide the result by g of sample per liter of prepared soln, 1.

(b) *Total sugars*.—Place in a 500 cc Erlenmeyer flask 150 cc of the clarified, deleadcd soln, 10, add 7.5 cc of HCl, and boil under a reflux condenser for exactly 1 hour. (If foaming occurs, add 5–10 drops of kerosene.) After boiling for an hour, remove the flask, stopper loosely when moderately cool, and let stand until ready for reduction, usually overnight. Cool the soln in ice  $H_2O$  for 20–30 min., add 2 drops of phenolphthalein soln, carefully neutralize with NaOH (1+1), and add HCl dropwise until the color of the indicator is just discharged. After bringing the soln to room temp., transfer it to a 200 cc flask, make to mark, mix, and filter until clear. Reduce Fehling's soln with duplicate 50 cc aliquots and calculate results as directed under 11(a).

(c) *Non-reducing sugars*.—The percentage of non-reducing sugars is the difference between Reducing Sugars, 11(a) and Total Sugars, 11(b).

DETECTION OF SULFITE-CELLULOSE<sup>3</sup>

## 12

## REAGENT

*Sulfite-cellulose soln*.—Dissolve 0.5 g of the total solids derived from sulfite-cellulose in 1 liter of  $H_2O$  and add sufficient tanning material, free from sulfite-cellulose, to give a concentration of 3.75–4.25 g of tannin per liter.

## 13

## DETERMINATION

Place 5 cc of the prepared tanning soln, 1, in a test tube. Add 0.5 cc of aniline and shake well; then add 2 cc of HCl and mix again. Compare the precipitate formed with that produced when the sulfite-cellulose soln is similarly treated. In the predetermined absence of the synthetic tanning materials known as syntans, sulfite-cellulose is held to be present if the volume of the precipitate approximately equals or exceeds that of the comparison soln.

## LIQUORS

## 14

## PREPARATION OF SOLUTION

Dilute the liquor with  $H_2O$  at room temp. to contain approximately 0.7 g of solids in 100 cc of soln. If the liquor does not give a proper soln with  $H_2O$  at room temp., dilute with  $H_2O$  at 80° and then cool to 20°, as directed under 1(a).

## 15

## TOTAL SOLIDS

Proceed as directed under 2.

## 16

## SOLUBLE SOLIDS

Proceed as directed under 5.

## 17

## NONTANNINS

Proceed as directed under 8, using the quantity of wet chromed hide powder that will give the ratio between the tannin and hide powder shown in the following table:<sup>9</sup>

TANNIN RANGE PER 100 cc	DRY HIDE POWDER PER 200 cc
<i>gram</i>	<i>gram</i>
0.35-0.45	9.0-11.0
0.25-0.35	6.5- 9.0
0.15-0.25	4.0- 6.5
0.00-0.15	0.0- 4.0

## TOTAL ACIDITY

## 18

## REAGENTS

(a) *Hematin indicator*.—Digest 0.5 g of hematin in 100 cc of cold neutral 95% alcohol.

(b) *Gelatin soln*.—Soak 10 g of gelatin in H<sub>2</sub>O at room temp. for 1-2 hours and then warm slightly, not exceeding 50°, to complete soln; add 25 cc of 95% alcohol, and dilute. If the gelatin soln is acid or alkaline, neutralize with 0.1 N NaOH or 0.1 N acetic acid, respectively, using hematin indicator, and dilute to 1 liter.

(c) *Kaolin*.—Digest with HCl (1+9), wash until it complies with the tests given under 3, dry, and preserve in a tightly stoppered bottle.

## 19

DETERMINATION<sup>10</sup>

Add 50 cc of the gelatin soln to 25 cc of the tanning liquor in a stoppered cylinder, dilute with H<sub>2</sub>O to 250 cc, add 15 g of the kaolin, and shake vigorously. Allow to settle for at least 15 min., remove 30 cc of the supernatant liquid, dilute with 50 cc of H<sub>2</sub>O, and titrate with 0.1 N NaOH, using the hematin indicator. 1 cc of 0.1 N NaOH = 0.2% of acid, calculated as acetic, in the liquor.

## RAW AND SPENT MATERIALS

(Under raw materials are included woods, barks, leaves, etc.)

## 20

## MOISTURE IN SAMPLE AS RECEIVED

Cut or break up large pieces and mix the sample rapidly to avoid change in moisture content. Dry as directed under 2, a suitable weighed quantity, dependent upon the physical condition and moisture content of the sample.

## 21

## PREPARATION OF SAMPLE

Dry the remainder of the sample at a temp. not above 60°, and grind to pass thru a 20-mesh sieve.

## 22

## MOISTURE IN PREPARED SAMPLE

Dry 10 g of the prepared sample, 21, as directed under 2, and calculate all results to an "as-received," "air-dried," or "moisture-free" basis, as desired.

## 23

## EXTRACTION

(a) *Woods, barks, and spent materials*.—Weigh a quantity of the sample that will give an extract containing as nearly as possible 4 g of tannin per liter. Transfer to a beaker and wet thoroly with hot H<sub>2</sub>O. Place a perforated porcelain plate in a tin-lined Cu extractor of the general form shown in Fig. 13; on the plate place a layer of cotton and wet thoroly with H<sub>2</sub>O. Connect the extractor with a 1000 cc Erlenmeyer flask (G) containing 800 cc of H<sub>2</sub>O, close stopcock (E), connect (D) by

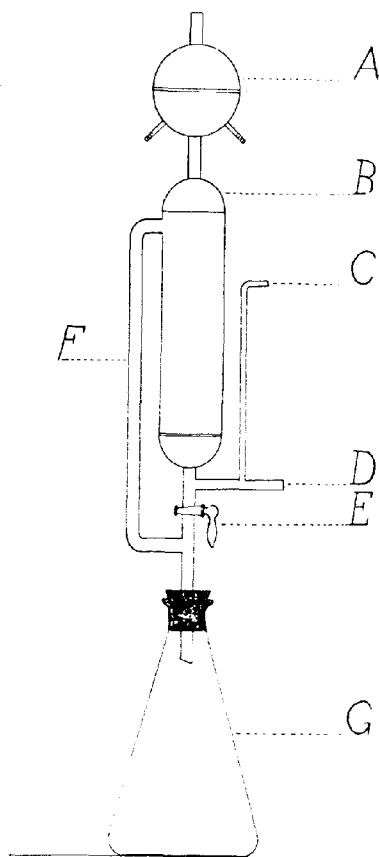


FIG. 13.—APPARATUS FOR EXTRACTING TANNING MATERIALS

materials, reduce the quantity of hide powder used in the determination of nontannins in accordance with the concentration of the soln and the schedule given under 17.

#### SELECTED REFERENCES

<sup>1</sup> The proceedings of the A.O.A.C. that deal with the early development of the methods of analysis of tanning materials will be found in Bureau of Chemistry Bulletins Nos. 43, 47, 49, 51, 56, 62, 67, 73, 81, and 90. These have been assembled in *J. Am. Leather Chem. Assoc.*, 15, ra-127 (1920).

<sup>2</sup> *J. A. Leather Chem. Assoc.*, 7, 288, 296 (1912).

<sup>3</sup> *Ibid.*, 1, 32 (1906); 9, 442 (1914).

<sup>4</sup> *Ibid.*, 10, 282 (1915).

<sup>5</sup> The official hide powder of the American Leather Chemists Association is prepared only by the Standard Manufacturing Company, Ridgway, Pa.

<sup>6</sup> *J. Am. Leather Chem. Assoc.*, 7, 292 (1912).

<sup>7</sup> *Ibid.*, 23, 91 (1928).

<sup>8</sup> *Ibid.*, 9, 36, 130 (1914).

<sup>9</sup> *Ibid.*, 5, 5 (1910).

<sup>10</sup> *Ibid.*, 3, 85 (1908); 4, 195 (1909).

a delivery tube to a 1000 cc graduated flask and close (D). Pour into the extractor the material to be extracted, washing it into the extractor with a minimum amount of hot  $H_2O$ . Open (D) and return the percolate thru the extractor 2 or 3 times until it is practically clear. Place a layer of cotton on top of the material. Connect the metal cap (B) and condenser (A) so that the condensate will drop onto the layer of cotton. Boil the water in (G) and collect 500 cc of percolate in the graduated flask in 2 hours. Then close (D), open (E), and continue the boiling for 14 hours, applying heat so that approximately 330 cc of  $H_2O$  will be condensed per hour. Transfer the extract in (G) to the graduated 1 liter flask, heat to  $80^\circ$ , cool as directed under 1(a) and dilute to the mark.

(b) *Materials other than woods, barks, and spent materials.*—Weigh a quantity of sample sufficient to give 2 liters of extract containing 4 g of tannin per liter. Place in the extractor described under (a), and digest for 1 hour with  $H_2O$  at room temp., at the end of which period start the extraction. Keepstopcock (E) closed and collect entirely thru the side tube (D) 2 liters of percolate in approximately 7 hours. Heat the percolate to  $80^\circ$ , cool as directed under 1(a), and dilute to the mark.

#### 24 ANALYSIS OF THE EXTRACT

Proceed as directed under 2–9, inclusive. With solns more dilute than specified, as is often the case with spent materials,

## XII. PLANTS

### 1 DIRECTIONS FOR SAMPLING<sup>1</sup>—OFFICIAL, FIRST ACTION

When more than one plant is sampled, include in the sample a sufficient number of plants to insure that it represents adequately the average composition of the entire lot of plants sampled. This number cannot be stated definitely; it will depend upon the variability in composition of the plants. Details of the procedure must be determined by the purpose for which the sample is taken.

### 2 PREPARATION OF SAMPLE<sup>1</sup>—OFFICIAL

(a) *For mineral constituents.*—Thoroughly remove all foreign matter from the material, especially adhering soil or sand, avoiding excessive washing to prevent leaching; air dry as rapidly as possible to prevent decomposition or loss in weight by respiration; grind; and preserve in tightly stoppered bottles. If the results are to be expressed on the fresh weight basis, record the weights of the sample before and after air drying. When determinations of Cu, Mn, Zn, Fe, Al, etc., are to be made, take precautions to prevent contamination of the sample by dust during air drying and from the grinding and sieving machinery.

(b) *For carbohydrates.*—Thoroughly remove all foreign matter and rapidly grind or chop the material into fine pieces. Add the weighed sample to sufficient hot redistilled 95% alcohol to which sufficient precipitated  $\text{CaCO}_3$  has been added to neutralize the acidity, using sufficient alcohol so that the final concentration, allowing for the  $\text{H}_2\text{O}$  content of the sample, will be approximately 80%. Heat close to the boiling point on a steam or water bath for 30 min., stirring frequently. The samples may be stored until needed for analysis.

### 3 MOISTURE—TENTATIVE

Determine as directed under **XXVII, 2, 6, or 7.**

### 4 ASH—TENTATIVE

Determine as directed under **XXVII, 8.**

### 5 SAND AND SILICA—OFFICIAL

Ignite 10–50 g of the substance in a flat-bottomed Pt dish in a muffle, at a temp. not exceeding dull redness, until the residue is white or nearly so. Moisten with 5 cc of  $\text{HCl}$ , evaporate to dryness, and heat on a steam bath for 3 hours to render the  $\text{SiO}_2$  insoluble. Moisten the residue with 5 cc of  $\text{HCl}$ , add about 50 cc of  $\text{H}_2\text{O}$ , heat on a water bath for a few minutes, filter thru a hardened filter, and wash thoroly. To this filtrate add the filtrate and washings from the alkali-soluble  $\text{SiO}_2$  determination (below) and dilute to 200 cc. Designate as soln A.

(a) *Sand.*—Wash the residue from the filter into a Pt dish and boil for about 5 min. with approximately 20 cc of a saturated soln of  $\text{Na}_2\text{CO}_3$ , add a few drops of a 10%  $\text{NaOH}$  soln, allow the mixture to settle, and decant thru an ignited and weighed Gooch crucible. Boil the residue in the dish with another 20 cc portion of the  $\text{Na}_2\text{CO}_3$  soln and decant as before. Repeat the process. Transfer the residue to the Gooch crucible and wash thoroly, first with hot  $\text{H}_2\text{O}$ , then with a little  $\text{HCl}$  (1+4), and finally with hot  $\text{H}_2\text{O}$  until free from chlorides. Dry the filter and contents, ignite, and weigh as sand. Confirm by microscopical examination.

(b) *Alkali-soluble  $\text{SiO}_2$ .*—Combine the alkaline filtrate and washings, acidify

with HCl, evaporate to dryness, add 5 cc of HCl, again evaporate, and dehydrate by heating to 110–120° for 2 hours. Moisten the residue with 5–10 cc of HCl, add about 50 cc of H<sub>2</sub>O, heat on a water bath 10–15 min., filter thru an ashless filter or an ignited and weighed Gooch crucible, wash with hot H<sub>2</sub>O, ignite, and weigh as SiO<sub>2</sub>. Add the filtrate to soln A.

#### 6 IRON AND ALUMINUM<sup>1</sup>—OFFICIAL, FIRST ACTION

Take an aliquot of soln A, 5, containing approximately 40 mg of Fe- and AlPO<sub>4</sub>. Oxidize the Fe. If the soln does not already contain an excess of phosphate, add to an aliquot containing approximately 40 mg of Fe- and AlPO<sub>4</sub>, 0.5 g of (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub>, stir until dissolved, and make up to 50 cc with H<sub>2</sub>O. Add a few drops of thymol blue and then add NH<sub>4</sub>OH until the soln just turns yellow. Run in 0.5 cc of HCl, follow with 25 cc of 25% NH<sub>4</sub> acetate, and stir. Let stand at room temp. until the precipitate settles (approximately 1 hour). Filter, and wash 10 times with hot 5% NH<sub>4</sub>NO<sub>3</sub> soln. Ignite, and weigh as Fe- and AlPO<sub>4</sub>.

Fuse the ignited precipitate in a Pt crucible with about 4 g of a mixture of equal parts of Na<sub>2</sub>CO<sub>3</sub> and K<sub>2</sub>CO<sub>3</sub>. When the fusion is complete, allow the crucible to cool, add 5 cc of H<sub>2</sub>SO<sub>4</sub>, and heat until copious fumes of SO<sub>3</sub> are given off. Cool, transfer to a flask, add H<sub>2</sub>O, and digest until the soln is clear. Reduce the Fe with Zn, cool, and titrate with 0.1 N KMnO<sub>4</sub> soln (cf. XXXVII, 59). Report as percentage of Fe<sub>2</sub>O<sub>3</sub>. Calculate the oxide to phosphate and subtract from total Fe- and AlPO<sub>4</sub>. This gives the AlPO<sub>4</sub>. Calculate to and report as Al<sub>2</sub>O<sub>3</sub>.

#### METHOD FOR IRON ONLY

##### 7 Colorimetric Method<sup>2</sup>—Official, First Action

To an aliquot of the soln containing approximately 0.2 mg of Fe, add H<sub>2</sub>O to make about 40 cc, 5 cc of HCl, and 0.3 cc of HNO<sub>3</sub>, and boil for about 30 min. Transfer the mixture to a 50 cc volumetric flask, add H<sub>2</sub>O to make about 35 cc, cool, add 10 cc of 20% K sulfoeyanate soln, fill to the mark, and compare the intensity of color with that of a standard containing somewhere near the same amount of Fe as the sample. Calculate the amount of Fe present.

##### 8 Titrimetric Method<sup>3</sup>—Tentative

Prepare the sample as directed under 7. Take a convenient aliquot of the soln, oxidize the Fe by adding dropwise a very dilute soln of KMnO<sub>4</sub> until a very, very faint permanganate color persists. Add 5 cc of 10% NH<sub>4</sub>CNS and titrate with approximately 0.02 N TiCl<sub>3</sub> to the disappearance of the red color. (Approximately a 0.02 N soln of TiCl<sub>3</sub> should be prepared, standardized with a known iron soln, kept in the dark in a well filled container and standardized against the Fe soln each time it is used or every few hours when making a large number of determinations. This is easily and quickly done if a standard iron soln is kept on hand.)

#### 9 MICRO METHOD FOR ALUMINUM ONLY<sup>4</sup>—TENTATIVE

Take an aliquot of soln A, 5, containing approximately 0.05 mg of Al. Oxidize the Fe by boiling with a few drops of HNO<sub>3</sub> and transfer to a conical centrifuge tube of about 25 cc capacity with marks at 15, 20, and 25 cc. If the quantity of Fe is very small, add Fe<sub>2</sub>Cl<sub>3</sub> soln equivalent to about 1 mg of iron; or if the quantity of phosphate present is small, add about 0.1 g of (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub> to insure complete precipitation of the Fe and Al. Dilute the contents to about 15 cc with H<sub>2</sub>O, neutralize with NH<sub>4</sub>OH, using a drop of dilute methyl red as indicator, and add 1 cc of a saturated soln of NH<sub>4</sub> acetate. Place the tube in a water bath until the precipitate

begins to settle (usually about 10 min.), centrifuge, decant, and discard the supernatant liquid. Dissolve the precipitate in 1 cc of approximately 6 *N* HCl with warming when necessary and dilute to 15 cc. Cool, add 1.25 cc of glacial acetic acid and 5 cc of 6 *N* NaOH (special Al-free), wash down the sides, and fill to the 25 cc mark. Let stand for about 1 hour and centrifuge. The precipitate contains the Fe and the soln the Al.

Transfer to a 50 cc volumetric flask as large an aliquot as can be drawn off. Add H<sub>2</sub>O to make about 20 cc, a small piece of litmus paper, and finally HCl (1+9) until the litmus paper just turns red. Determine the Al as follows: Add 5 cc of 5 *N* NH<sub>4</sub> acetate, 5 cc of 1.5 *N* HCl, and 2 cc of 0.1% of the dye Aluminon (ammonium salt of aurintricarboxylic acid) and place in a water bath at about 80° for 10 min. Add 5 cc of 5 *N* NH<sub>4</sub>Cl, cool to room temp., add 5 cc of 3.2 *N* (NH<sub>4</sub>)<sub>2</sub>CO<sub>3</sub> while shaking gently, fill to the mark with H<sub>2</sub>O, and mix. At this point the reaction should be 7.1-7.3 and the red color of a blank should disappear in about 15 min.. (The exact concentration of the reagents is not important, but the final pH is, and the amount of (NH<sub>4</sub>)<sub>2</sub>CO<sub>3</sub> necessary to bring the soln to the above pH should be determined by neutralizing similar solns without adding the dye.) Simultaneously with the above procedure run a standard (or standards if necessary) containing a given quantity of Al. After allowing the mixture to stand for 20 min. for the excess dye to decolorize, compare the color intensities and read the amount of Al from a curve plotted as described in the following paragraph.

If only a small number of determinations are to be made, prepare 4 standards containing 0.01, 0.03, 0.05 and 0.07 mg of Al, respectively, and run these with the samples. Compare all these solns with the standard containing 0.03 mg of Al and calculate the results to a colorimeter reading of 30 for this standard. Arbitrarily give 0.005 mg of Al a reading of 100 and with this and the 4 readings on the standards plot a curve. Read the quantity of Al in each sample from this curve. If a large number of determinations are to be made, extending over a period of time, it is advisable to make determinations on several series of standards and plot a curve from the average of these results. It is then necessary to run only 1 standard each time determinations are to be made, and the results can be read from the curve. Blanks must be run on both the Fe and Al determinations as nearly all the reagents contain traces of these elements.

#### CALCIUM—OFFICIAL

##### 10

##### *Macro Method<sup>b</sup>*

(Applicable to material containing less than 0.05% of MnO.)

Transfer an aliquot of soln A, 5, to a 200 cc beaker, add H<sub>2</sub>O if necessary to make to 50 cc, heat to boiling, add 10 cc of saturated soln of NH<sub>4</sub>C<sub>2</sub>O<sub>4</sub> and a drop of methyl red. Almost neutralize with NH<sub>4</sub>OH and boil until the precipitate is coarsely granular. Cool, add NH<sub>4</sub>OH (1+4) until the color is a faint pink (pH 5.0) and allow to stand at least 4 hours. Filter and wash with H<sub>2</sub>O at room temp. until the filtrate is free from oxalates. Break the point of the filter with a Pt wire and wash the precipitate into the beaker in which the Ca was precipitated with hot H<sub>2</sub>SO<sub>4</sub> (1+4) and hot water. Add about 10 cc of H<sub>2</sub>SO<sub>4</sub> (1+4), heat to about 90°C., add about 50 cc of hot H<sub>2</sub>O and titrate with 0.05 *N* KMnO<sub>4</sub>. Finally add the filter paper to the soln and complete the titration.

##### 11

##### *Micro Method<sup>b</sup>*

Ignite 2 g of the substance in a small crucible in a muffle at dull red heat. Dissolve the ash in HCl (1+4) and transfer to a 100 cc beaker. Add 5 cc of HCl and evaporate



to dryness on the steam bath to dehydrate the  $\text{SiO}_2$ . Moisten the residue with 5 cc of  $\text{HCl}$ , add about 50 cc of distilled  $\text{H}_2\text{O}$ , heat for a few minutes on the steam bath, transfer to a 100 cc volumetric flask, cool quickly to room temp., make to volume, shake, and filter, discarding the first portion of the filtrate. Pipet a 15 cc aliquot into a conical-tipped centrifuge tube containing 2 cc of saturated  $\text{NH}_4$  oxalate soln and 2 drops of 0.05% methyl red. Add 2 cc of acetic acid (1+4), rotating the tube to mix its contents thoroly. Add, while intermittently rotating the tube,  $\text{NH}_4\text{OH}$

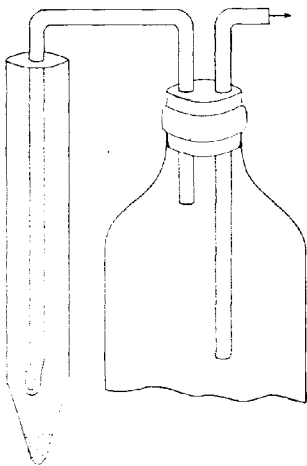


FIG. 14.—SUCTION DEVICE USED IN MICRO METHOD FOR THE DETERMINATION OF CALCIUM

(1+4) until the soln is faintly alkaline, after which add a few drops of dilute acetic acid (1+4) with a dropper until the color is adjusted to a faint pink (pH 5.0). (It is important at this point to rotate the tube so that the last bit of liquid in the conical tip is the color required.) Allow the mixture to stand at least 4 hours and whirl the tube in the centrifuge for 15 min. (The precipitate should then be in a firm lump in the tip of the tube.) Remove the supernatant liquid by means of the suction device (Fig. 14), taking care not to disturb the precipitate. Wash the precipitate by adding 2 cc of  $\text{NH}_4\text{OH}$  (1+49), rotating the tube to break up the precipitate. (It may be necessary to jar the tube sharply.) Return the tube to the centrifuge for 10 min., and again remove the supernatant liquid and wash with the reagent as before. Repeat this operation until the precipitate has been washed 3 times. When the supernatant liquid has been removed after the final centrifuging add 2 cc of  $\text{H}_2\text{SO}_4$  (1+4) to the tube, break up the precipitate as before, heat on the steam

bath to between 80 and 90°, and titrate in the tube with 0.02  $N$   $\text{KMnO}_4$ , rotating the liquid during the titration to attain a proper end point. If the tube cools below 60° during the addition of the  $\text{KMnO}_4$ , reheat it in the steam bath for a few minutes and complete the titration. Run a blank on an identical amount of  $\text{H}_2\text{SO}_4$  in a similar tube heated to the same temp. to determine the quantity of 0.02  $N$   $\text{KMnO}_4$  necessary to give the color of the end point. Subtract this value from the buret reading. 1 cc of 0.02  $N$   $\text{KMnO}_4$  = 0.0004 g of Ca. Report as percentage of Ca.

## 12

## MAGNESIUM—OFFICIAL

To the combined filtrate and washings from the Ca determination, 10, add 30 cc of  $\text{HNO}_3$  and evaporate to dryness to decompose the  $\text{NH}_4$  salts. Take up with 5 cc of  $\text{HCl}$  and make to a volume of about 100 cc with  $\text{H}_2\text{O}$ . Add 5 cc of a 10% Na citrate soln and 10 cc of a 10%  $(\text{NH}_4)_2\text{HPO}_4$  soln, or enough to precipitate all the Mg. Add  $\text{NH}_4\text{OH}$  (1+4) with constant stirring (using a rubber policeman) until the soln is faintly alkaline and the precipitate forms; then add about 5 cc of  $\text{NH}_4\text{OH}$ , stir vigorously until the precipitate is granular, and allow to stand in a cool place overnight. Filter and wash free from chlorides with cold  $\text{NH}_4\text{OH}$  (1+10). Ignite, and weigh as  $\text{Mg}_2\text{P}_2\text{O}_7$ . Calculate and report as percentage of  $\text{MgO}$ .

## 13.

## MANGANESE—OFFICIAL

To an aliquot of soln A, 5, representing about 0.2–0.5 g of ash, add 15 cc of  $\text{H}_2\text{SO}_4$  and evaporate to about 30 cc. Add 5–10 cc of  $\text{HNO}_3$  and continue the evaporation. (It is neither necessary nor advisable to evaporate until dense fumes appear, since the  $\text{Fe}_2(\text{SO}_4)_3$  then dissolves with difficulty.  $\text{HNO}_3$  may be present, but not  $\text{HCl}$ .) Add  $\text{H}_2\text{O}$ , a little at a time; heat until the Fe salts have dissolved; and dilute to about 150 cc. Add 0.3 g of  $\text{KIO}_4$  in small portions, boil for a few minutes or until the color of the  $\text{KMnO}_4$  shows no further increase in intensity, and allow to cool.

Prepare the standard as follows: To a volume of  $\text{H}_2\text{O}$  equal to the sample add 15 cc of  $\text{H}_2\text{SO}_4$  and sufficient pure  $\text{Fe}(\text{NO}_3)_3$ , free from Mn, to equal approximately the quantity of Fe in the sample. Add a measured quantity of 0.1 N  $\text{KMnO}_4$  soln until the color is slightly darker than the sample, and then add 0.3 g of  $\text{KIO}_4$  and boil for a few minutes. When cool, transfer the sample and standard to 250 cc flasks and dilute to the mark with  $\text{H}_2\text{O}$ . If the color is weak, it may be necessary to dilute to less than 250 cc. Compare the colors in any standard colorimeter. Calculate and report the results as percentage of  $\text{Mn}_2\text{O}_3$ .

## 14

## SODIUM AND POTASSIUM—OFFICIAL

Moisten 1–10 g of the sample with  $\text{H}_2\text{SO}_4$  (1+10), dry in an oven, and ignite in a muffle at a low red heat to destroy the organic matter. Heat the residue on a steam bath with 2–5 cc of  $\text{HCl}$  and about 50 cc of  $\text{H}_2\text{O}$ . Transfer to a beaker and add  $\text{NH}_4\text{OH}$  dropwise until the precipitate formed requires several seconds to dissolve, thus leaving the soln but faintly acid. Heat nearly to boiling, and add  $\text{NH}_4\text{OH}$  to precipitate all the Fe, Al, etc. Boil in a covered beaker for about 1 min.; remove, and if no  $\text{NH}_3$  is detected by smelling, continue the addition, dropwise, until it can be detected. Do not allow the precipitate to settle, but stir and pour on the filter. Wash immediately with hot  $\text{H}_2\text{O}$ , using, to effect rapid filtration, a fine jet directed around the edge of the precipitate to cut it free from the paper. Wash the precipitate several times, return it to the original beaker, dissolve with a few drops of  $\text{HCl}$ , and warm. Reprecipitate the Fe, Al, and  $\text{P}_2\text{O}_5$  with  $\text{NH}_4\text{OH}$  as directed above; filter and wash until free from chlorides. Evaporate the combined filtrates and washings to dryness, heat below redness until  $\text{NH}_4$  salts are expelled, and dissolve in hot  $\text{H}_2\text{O}$ . Add 5 cc of a saturated soln of  $\text{Ba}(\text{OH})_2$ , heat to boiling, allow to settle a few minutes, and determine whether or not the precipitation is complete by the addition of more of the  $\text{Ba}(\text{OH})_2$  soln to a little of the clear liquid. When no further precipitate is produced, filter and wash thoroly with hot  $\text{H}_2\text{O}$ . Heat the filtrate to boiling and add  $\text{NH}_4\text{OH}$  (1+4) and a 10%  $(\text{NH}_4)_2\text{CO}_3$  soln to complete the precipitation of the Ba, Ca, etc. Let stand a short time on a water bath, filter, and wash the precipitate thoroly with hot  $\text{H}_2\text{O}$ . Evaporate the filtrate and washings to dryness, expel  $\text{NH}_4$  salts by heating below redness, treat with a little hot  $\text{H}_2\text{O}$ , and add a few drops of the dilute  $\text{NH}_4\text{OH}$ , 1 or 2 drops of the  $(\text{NH}_4)_2\text{CO}_3$  soln, and a few drops of a saturated soln of  $\text{NH}_4$  oxalate. Let stand for a few minutes on a water bath and set aside for a few hours. Filter, evaporate to complete dryness on a water bath, and heat at a temp. not exceeding dull redness until all  $\text{NH}_4$  salts are expelled and the residue is nearly or quite white. Dissolve in a minimum quantity of  $\text{H}_2\text{O}$ , filter into a weighed Pt dish, add a few drops of  $\text{HCl}$ , evaporate to dryness on a water bath, heat at a temp. not exceeding dull redness, cool in a desiccator, and weigh as  $\text{KCl}$  plus  $\text{NaCl}$ . Repeat the heating until constant weight is obtained.

**15** *Platinic Chloride Method for Potassium—Official*

Dissolve the residue with a few cc of  $H_2O$ , acidify with a few drops of  $HCl$ , add an excess of  $PtCl_4$  soln, II, 42(b), and proceed as directed under II, 47, beginning with "Evaporate on a water bath to a thick paste. . . ."  $K_2PtCl_6 \times 0.16084 = K$ . If it is desired to determine the Na, calculate the K to  $KCl$  and subtract this from the  $KCl + NaCl$  found in the preceding paragraph.

**16** *Perchloric Acid Method for Sodium and Potassium<sup>2</sup>—Tentative*

Prepare the material as directed in 14 until the heavy metals have been removed and the two elements are in the form of chlorides. (Sulfates must be absent.) Add 3–5 cc of 60%  $HClO_4$ . Evaporate to dryness, dissolve in hot  $H_2O$ , and again evaporate to dryness. Heat to  $350^\circ$ , cool, and weigh if it is desired to obtain the combined perchlorates. Add 10–20 cc of a mixture of anhydrous ethyl acetate and C.P. normal butyl alcohol in equal proportions by volume. Digest near the boiling point for several minutes. Decant into a Gooch crucible. Wash once or twice by decantation with a few cc of the acetate-alcohol mixture. Dissolve in the minimum quantity of  $H_2O$ , evaporate to dryness, and extract as before. Filter, and wash several times with 1 cc of the acetate-alcohol mixture. Dry in an oven at  $110^\circ$  for several minutes and heat at  $350^\circ$  for 15 min. Cool and weigh.  $KClO_3 \times 0.28218 = K$ . Calculate the Na by difference.

**17** *Rapid Method for Potassium Only—Official*

Proceed as under 14 to "and if no  $NH_3$  is detected . . . until it can be detected." Then add a few cc of saturated  $(NH_4)_2C_2O_4$ , let stand for a few hours, filter into a Pt evaporating dish, evaporate to drive off the excess of  $NH_3$ , add 0.5 cc of  $H_2SO_4$  (1+1), evaporate, ignite by whirling the dish over a free flame, and proceed as directed under 15.

*Uranyl Acetate Method for Sodium Only<sup>2</sup>—Tentative***18**

## REAGENT

*Magnesium uranyl acetate soln.*

(a) *Crystallized uranyl acetate.*—To 85 g add 60 g of glacial acetic acid and  $H_2O$  to make 1000 cc.

(b) *Crystallized magnesium acetate.*—To 500 g add 60 g of glacial acetic acid and water to make 1000 cc.

Heat (a) and (b) separately to about  $70^\circ$  until all the salts are dissolved and then mix the two solns at this temp. and allow to cool to  $20^\circ$ . Place the vessel containing the mixed reagent in  $H_2O$  at  $20^\circ$ , and hold at this temp. for an hour or two until the slight excess of salts is crystallized out. Filter the reagent thru a dry filter into a dry bottle.

**19**

## DETERMINATION

Moisten 1–10 g of the sample with  $H_2SO_4$  (1+10), dry in an oven, and ignite in a muffle at a low red heat to destroy the organic matter. Heat the residue on a steam bath with 2–5 cc of  $HCl$ , add about 40 cc of  $H_2O$ , and heat to boiling. Add a sufficient amount of  $CaCl_2$  soln to precipitate all the phosphates. Precipitate the phosphates by making slightly alkaline with  $NH_4OH$ . Filter, and evaporate to 5 cc or less if no salts separate. Cool. Add 100 cc of the magnesium uranyl acetate, place the mixture in a water bath at  $20^\circ$  and either stir vigorously for 45 min. or let stand for

24 hours. Filter with suction and wash with 95% alcohol saturated with the Na-Mg-uranyl acetate. Dry at 105–110° for 30 min., cool, and weigh. Weight of Na-Mg-uranyl acetate  $\times 0.0153 = \text{Na}$ .

#### COPPER<sup>10</sup>—OFFICIAL, FIRST ACTION

20

##### REAGENTS

(a) *Potassium ethyl xanthate*.—0.1% water soln prepared fresh each time it is used.

(b) *Standard copper sulfate*.—Dissolve 0.3928 g of pure  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  in  $\text{H}_2\text{O}$ , dilute in a volumetric flask to 1000 cc, and mix. 1 cc = 0.0001 g of Cu.

(c) *Filter-paper pulp*.—Moisten and tear a good grade of sheet filter paper into bits and place in a porcelain dish. Add, while stirring with a glass rod, enough cold HCl to disintegrate the paper and reduce the mass to a mushy consistency. Transfer the pulp to a large Büchner funnel and wash free of acid, using suction. Transfer the washed pulp into a clean glass bottle and add  $\text{H}_2\text{O}$  to make a thick pulp suitable for making a pad in a Caldwell crucible. (The precipitates of Cu and Zn obtained in these methods can be readily filtered and washed upon pads made with this filter-paper pulp by the use of the suction pump.)

21

##### DETERMINATION

Ash 100–500 g of the finely divided air-dried plant material in  $\text{SiO}_2$  dishes with a small flame, but do not allow the plant material to burn with a blaze. After the volatile matter has been dispelled, complete the ashing in a muffle furnace maintained at the temp. of a faint red glow. Hasten the ashing process by removing the dishes from the muffle at intervals and breaking up the lumps with a Pt stirring rod. After the C has been oxidized as completely as possible, cool the dish, moisten the ash with  $\text{H}_2\text{O}$ , wash into a 250 cc beaker, and cover with a watch-glass. Decompose the ash with HCl (1+1) introduced thru the lip of the beaker beneath the watch-glass by means of a pipet. After effervescence has ceased, rinse the watch-glass into the beaker, filter the insoluble residue out on a Büchner funnel, and wash free of chlorides. If the insoluble residue contains undecomposed particles of C, transfer it into a  $\text{SiO}_2$  dish and reignite in the muffle furnace until all particles of C are decomposed and a light colored ash remains. Redigest the ash on a hot water bath with 15 cc of HCl (1+1), filter, and wash free of chlorides. Combine the filtrates in a clean porcelain dish, evaporate to dryness, and bake at 110° until the HCl is expelled. Moisten the dry residue with 10 cc of HCl (1+1) and digest, with stirring, for 10 min. Dilute with hot  $\text{H}_2\text{O}$ , filter out the  $\text{SiO}_2$ , wash free of chlorides, combine with the insoluble residue, ignite, and weigh. Make the filtrate to about 250 cc, heat to near the boiling point, and pass a slow stream of  $\text{H}_2\text{S}$  thru the soln for 15 min. Rinse the  $\text{H}_2\text{S}$  delivery tube into the flask, tightly stopper, and set aside until the precipitate settles and the supernatant soln is clear. Filter the  $\text{CuS}$  on a pad of the paper pulp and wash with HCl (0.25 N) saturated with  $\text{H}_2\text{S}$ ; ignite in a porcelain crucible and dissolve the  $\text{CuO}$  in a few drops of  $\text{HNO}_3$  (1+9) and one drop of HCl (1+9). Filter the soln and wash the filter paper clean. Evaporate the soln to dryness in a porcelain dish 3 times with the addition of a few drops of  $\text{HNO}_3$  (1+9) and take up with a very small drop of the  $\text{HNO}_3$  delivered from a stirring rod having a sharp point. Make to a volume of 50 cc, and transfer an aliquot of 5 cc to a Nessler tube containing 10 cc of the K ethyl xanthate. Mix the solns and dilute to 25 cc. Transfer 10 cc of the K ethyl xanthate to a second Nessler tube, dilute to a volume of about 15 cc, and add the standard Cu soln, dropwise with thoro

mixing with a glass stirring rod until the color in the standard tube apparently matches the color in the tube containing the sample. Make the volume and the final adjustment of the color of the standard in the tube containing it. Record the number of cc of the standard Cu soln required and calculate the percentage of Cu.

To the filtrate from the CuS add 5 cc of  $\text{HNO}_3$  (1 + 1) and boil for 10 min. to oxidize the remaining metals. Cool the soln and make to a convenient volume (250 cc). From this stock soln take suitable aliquots for the determination of Zn or other elements contained in the ash of the sample.

#### ZINC<sup>10</sup>—OFFICIAL, FIRST ACTION

##### 22

##### REAGENTS

(a) *Potassium ferrocyanide soln.*—Dissolve 2 g in  $\text{H}_2\text{O}$  and dilute to 100 cc. Should be freshly prepared.

(b) *Zinc sulfate.*—Dissolve 1 g of C.P. Zn in  $\text{H}_2\text{SO}_4$  and dilute to 1000 cc. 1 cc = 0.001 g of Zn.

##### 23

##### DETERMINATION

Transfer an aliquot equivalent to 25 g of plant material from the stock soln (filtrate from the Cu determination—last paragraph under 21) to a 250 cc Erlenmeyer flask and add  $\text{NH}_4\text{OH}$  in slight excess. Dissolve the precipitate in a slight excess of pure glacial acetic acid, saturate the soln with  $\text{H}_2\text{S}$ , and set aside for several hours for the precipitate to settle. The acidity of the soln must be kept between pH 2 and pH 3, and the presence of a citrate helps to prevent the precipitation of Fe and Mn. Hence at this point add about 2 g of citric acid, ammonia until neutral to methyl orange, and then 10 cc of a formic acid soln (containing in 100 cc of the mixture, 3 cc of  $\text{NH}_4\text{OH}$ , 20 cc of 90% formic acid, and 25 g of  $(\text{NH}_4)_2\text{SO}_4$ ). Filter on a pad of paper pulp, 20(c), and wash with a soln of acetic acid (1 + 10) containing 10 g of  $\text{NH}_4$  acetate in 100 cc of soln, saturated with  $\text{H}_2\text{S}$ . Ignite the pad of paper pulp and precipitate in a porcelain crucible, cool, dissolve the residue in a few drops of  $\text{HCl}$  (1 + 9) and warm on the hot water bath. Transfer to a 100 cc beaker and add  $\text{NH}_4\text{OH}$  in slight excess. Heat on the water bath for 5 min., filter, and wash the precipitate. Add acetic acid in slight excess to the filtrate and saturate the hot soln with  $\text{H}_2\text{S}$ ; stopper tightly and set aside several hours in a warm place for the precipitate to settle; filter; wash as previously described and ignite in a porcelain crucible. Dissolve the ignited residue of  $\text{ZnO}$  in 10 cc of 0.1 N  $\text{H}_2\text{SO}_4$ , make to a volume of 50 cc, and mix. Transfer an aliquot of 5 cc to a 50 cc Nessler tube containing 5 cc of the K ferrocyanide. Dilute to 50 cc, mix with a stirring rod, and let stand 5–10 min. To another Nessler tube containing 5 cc of the K ferrocyanide diluted to about 40 cc, add dropwise with stirring the Zn sulfate until the turbidity in the standard matches the turbidity of the sample. From the number of cc of the Zn standard required calculate the percentage of Zn contained in the sample.

#### ARSENIC—TENTATIVE

##### 24

##### PREPARATION OF SOLUTION

Proceed as directed under **XXIX, 3**.

##### 25

##### DETERMINATION

Proceed as directed under **XXIX, 4**, or take an aliquot and determine as directed under **VI, 12**, beginning with "add 3 cc of  $\text{H}_2\text{SO}_4$ ."

## SULFUR

*Sodium Peroxide Method*<sup>11</sup>—Official

26

## PREPARATION OF SOLUTION

Place 1.5–2.5 g of material in a Ni crucible of about 100 cc capacity and add 5 g of anhydrous  $\text{Na}_2\text{CO}_3$ . Mix thoroly, using a Ni or Pt rod, and moisten with approximately 2 cc of  $\text{H}_2\text{O}$ . Add  $\text{Na}_2\text{O}_2$ , approximately 0.5 g at a time, thoroly mixing the charge after each addition, and continue until the mixture becomes nearly dry and quite granular. (About 5 g of  $\text{Na}_2\text{O}_2$  is required.) Place the crucible over a S-free flame or an electric hot plate and heat carefully, with occasional stirring, until the contents are fused. (If the material ignites, the determination is worthless.) After fusion, remove the crucible, allow to cool somewhat, and cover the hardened mass with more of the  $\text{Na}_2\text{O}_2$  to a depth of about 0.5 cm. Heat gradually and finally with full flame until fusion again takes place, rotating the crucible from time to time in order to bring any particles adhering to the sides into contact with the oxidizing material. Continue the heating for 10 min. after fusion is complete. Cool somewhat, place the warm crucible and contents in a 600 cc beaker, and carefully add about 100 cc of  $\text{H}_2\text{O}$ . After the initial violent action has ceased, wash the material out of the crucible, make slightly acid with HCl (adding small portions at a time), transfer to a 500 cc flask, cool, and dilute to volume. Filter, and determine sulfates in an aliquot of the filtrate as directed under 27.

27

## DETERMINATION

Add  $\text{H}_2\text{O}$  to make the aliquot to about 200 cc and add HCl to make approximately 0.5 cc of free acid present. Heat to boiling and add 10 cc of 10%  $\text{BaCl}_2$  soln dropwise with constant stirring. Continue the boiling for about 5 min. and allow to stand for 5 hours or longer in a warm place. Decant the liquid thru an ashless filter or an ignited and weighed Gooch crucible, treat the precipitate with 15–20 cc of boiling  $\text{H}_2\text{O}$ , transfer to the filter, and wash with boiling  $\text{H}_2\text{O}$  until the filtrate is free from chlorides. Dry the precipitate and filter, ignite, and weigh as  $\text{BaSO}_4$ . Multiply the result by the factor 0.13736 and report as percentage of S.

*Magnesium Nitrate Method*<sup>12</sup>—Official

28

## PREPARATION OF SOLUTION

Weigh 1–5 g of material into a large porcelain or Sillimanite crucible. Add 7.5 cc of  $\text{Mg}(\text{NO}_3)_2$  soln, II, 7(e), taking care that all the material is brought in contact with the soln, and heat on an electric hot plate ( $180^\circ$ ) until no further action takes place. Transfer the crucible while hot to an electric muffle and allow it to remain at low heat (muffle must not show any red) until the charge is thoroly oxidized. (No black particles should remain. It may be necessary to break up the charge and return to the muffle.) Remove the crucible from the muffle and allow to cool. Add  $\text{H}_2\text{O}$ , then HCl in excess. Bring the soln to a boil, filter, and wash thoroly. If preferred, transfer the soln to a 250 cc volumetric flask before filtering and make to the mark with  $\text{H}_2\text{O}$ .

29

## DETERMINATION

Dilute the entire filtered soln, 28, to 200 cc or take an aliquot of 100 cc of the measured volume, make to 200 cc, and proceed as directed under 27.

PHOSPHORUS<sup>23</sup>

30

*Macro Method—Official*

(a) *For samples exceedingly high in P and low in Ca and Mg, such as certain seeds, grains, etc.*—Prepare as directed under 28, or evaporate the filtrate and washings from the S determination, 27, to 50 cc and proceed as directed under II, 9 or 12.

(b) *For all other samples.*—Take a 50 cc aliquot of soln A, under 5, and proceed as directed under II, 9 or 12.

*Micro Method<sup>14</sup>—Official*

31

## REAGENTS

(a) *Standard potassium dihydrogen phosphate soln.*—Dissolve 0.4394 g of pure dry  $\text{KH}_2\text{PO}_4$  in distilled  $\text{H}_2\text{O}$  and make up to a liter. 50 cc of this soln diluted to 200 cc gives a standard of which 2 cc = 0.05 mg of P.

(b) *Ammonium molybdate soln.*—Dissolve 25 g of  $\text{NH}_4$  molybdate in 300 cc of  $\text{H}_2\text{O}$ . Dilute 75 cc of  $\text{H}_2\text{SO}_4$  to 200 cc and add to the  $\text{NH}_4$  molybdate soln.

(c) *Hydroquinone soln.*—Dissolve 0.5 g of hydroquinone in 100 cc of distilled  $\text{H}_2\text{O}$ , and add one drop of  $\text{H}_2\text{SO}_4$  to retard oxidation.

(d) *Sodium sulfite soln.*—Dissolve 200 g of  $\text{Na}_2\text{SO}_3$  in distilled  $\text{H}_2\text{O}$ , make up to a liter, and filter. Either keep this soln well-stoppered or make it up fresh each time.

(e) *Magnesium nitrate soln.*—Dissolve 160 g of  $\text{MgO}$  in  $\text{HNO}_3$  (1+1), avoiding an excess of the acid; add a little  $\text{MgO}$  in excess, boil, filter from the excess  $\text{MgO}$ ,  $\text{Fe}_2\text{O}_3$ , etc., and dilute to 1 liter.

32

## PREPARATION OF SOLUTION

To 1 or 2 g of the substance in a small Sillimanite crucible add 1 cc of the  $\text{Mg}(\text{NO}_3)_2$  soln, and place on the steam bath. After a few minutes cautiously add a few drops of  $\text{HCl}$ , taking care that the formation of gas bubbles does not push portions of the sample over the edge of the crucible. Make 2 or 3 further additions of a few drops of  $\text{HCl}$  while the sample is on the bath so that as it approaches dryness there is a tendency for it to char. If the contents of the crucible become so viscous that no further drying may be obtained on the bath, complete the drying on a hot plate, put on a crucible cover, transfer to a cold muffle, and ignite at dull red heat for 6 hours or until an even grey ash is obtained. (It may be necessary to cool the crucible, dissolve the ash in a little  $\text{H}_2\text{O}$  or alcoholic glycerol, evaporate to dryness, and return uncovered to the muffle for another 4 or 5 hours.) Cool, take up with  $\text{HCl}$  (1+4), and transfer to a 100 cc beaker. Add 5 cc of  $\text{HCl}$  and evaporate to dryness on the steam bath to dehydrate the  $\text{SiO}_2$ . Moisten the residue with 2 cc of  $\text{HCl}$ , add about 50 cc of distilled  $\text{H}_2\text{O}$ , heat for a few minutes on the bath, transfer to a 100 cc volumetric flask, cool immediately, make to volume, and filter, discarding the first portion of the filtrate.

33

## DETERMINATION

To a 5 cc aliquot of the filtrate in a 10 cc volumetric flask add 1 cc of the  $\text{NH}_4$  molybdate, rotate the flask to mix, and allow to stand a few moments. Add 1 cc of the hydroquinone soln, again rotate the flask, and add 1 cc of the  $\text{Na}_2\text{SO}_3$  soln. (These last 3 additions may be made with a Mohr pipet.) Make to volume with distilled  $\text{H}_2\text{O}$ , stopper the mouth of the flask with the thumb or forefinger, and shake to mix the contents thoroly. Allow to stand 30 min. and compare immediately in a colorimeter with 2 cc of the standard  $\text{KH}_2\text{PO}_4$  soln treated simultaneously and

in an identical manner. With either the unknown or standard set at 25.0 mm, readings within 10 mm (i.e., a range of 20 mm) are accurate. If the concentration of P in the unknown set is outside this range, it may be brought nearer to that of the standard by diluting the filtrate, ashing a smaller or larger sample, making the filtrate to a smaller or larger volume, or using a smaller aliquot. Report as percentage of P.

## CHLORINE

34

## PREPARATION OF SOLUTION—OFFICIAL

Moisten 5 g of the substance in a Pt dish with 20 cc of a 5%  $\text{Na}_2\text{CO}_3$  soln, evaporate to dryness, and ignite as thoroly as possible at a temp. not exceeding dull redness. Extract with hot  $\text{H}_2\text{O}$ , filter, and wash. Return the residue to the Pt dish and ignite to an ash; dissolve in  $\text{HNO}_3$  (1+4), filter from any insoluble residue, wash thoroly, and add this soln to the  $\text{H}_2\text{O}$  extract.

35

*Gravimetric Method—Official*

To the prepared soln add a 10%  $\text{AgNO}_3$  soln, avoiding more than a slight excess. Heat to boiling, protect from the light, and allow to stand until the precipitate is coagulated. Filter on a weighed Gooch crucible, previously heated to 140–150°, and wash with hot  $\text{H}_2\text{O}$ , testing the filtrate to prove excess of  $\text{AgNO}_3$ . Dry the  $\text{AgCl}$  at 140–150°, cool, and weigh. Report as percentage of Cl.

*Volumetric Method I<sup>15</sup>—Official*

36

## REAGENTS

(a) *Silver nitrate soln.*—Adjust to exact 0.1 N strength by standardizing against a 0.1 N  $\text{NaCl}$  soln containing 5.846 g of pure  $\text{NaCl}$  per liter.

(b) *Ammonium or potassium thiocyanate.*—0.1 N. Adjust by titrating against the 0.1 N  $\text{AgNO}_3$ .

(c) *Ferric indicator.*—A saturated soln of ferric ammonium alum.

(d) *Nitric acid.*—Free from lower oxides of N by diluting the usual pure acid with about  $\frac{1}{4}$  volume of  $\text{H}_2\text{O}$ , and boiling until perfectly colorless.

37

## DETERMINATION

To the soln prepared as directed under 34, add a known volume of the 0.1 N  $\text{AgNO}_3$  in slight excess. Stir well, filter, and wash the  $\text{AgCl}$  precipitate thoroly. To the combined filtrate and washings add 5 cc of the ferric indicator and a few cc of the  $\text{HNO}_3$  and titrate the excess of Ag with the 0.1 N thiocyanate until a permanent light brown color appears. From the number of cc of 0.1 N  $\text{AgNO}_3$  used, calculate the quantity of Cl. 1 cc of 0.1 N  $\text{AgNO}_3$  = 0.00355 g of Cl.

*Volumetric Method II<sup>16</sup>—Tentative*

(If bromides or iodides are present in significant quantities the results must be corrected accordingly.)

38

## REAGENTS

(a) *Standard potassium iodide soln.*—Weigh out 4.6826 g of the pure, dried salt, dissolve in  $\text{H}_2\text{O}$ , and dilute to 1 liter. 1 cc = 1 mg of Cl.

(b) *Approximately 0.3 N silver nitrate soln.*—Dissolve 48 g of the salt in  $\text{H}_2\text{O}$ , filter, and dilute to 1 liter. 1 cc = 10 mg of Cl (approximately).

(c) *Standard silver nitrate soln.*—Dilute 100 cc of Reagent (b) to about 900 cc and adjust by standardizing against Reagent (a) so that 1 cc = 1 mg of Cl.



(d) *Chlorine-free starch indicator*.—For each 100 cc of the final soln take 2.5 g of soluble starch and make to a paste with cold  $H_2O$ . After stirring out the lumps, add 25–50 cc more cold  $H_2O$  and stir or shake for 5 min. Centrifuge, decant, and discard the liquid. Repeat the extraction 3 times and finally transfer the residue to a flask containing the proper quantity of boiling  $H_2O$ . Stir again, allow to come to a boil, cover with a small beaker, and cool under the tap, shaking occasionally.

(e) *Dilute sulfuric acid soln*.—Add 35 cc of  $H_2SO_4$  to 1 liter of  $H_2O$  and cool to room temp.

(f) *Iodine indicator*.—To approximately 20 g of pure I in a 500 cc ground-glass-stoppered bottle add 400 cc of Reagent (e) and shake for 10 min. Decant, and discard the first soln since it may contain iodides. Repeat the process and store this soln in small ground-glass-stoppered bottles.

(g) *Potassium permanganate*.—Dissolve 60 g of the salt in 400 cc of warm  $H_2O$  (about  $50^\circ$ ) and dilute to 1 liter.

(h) *Potassium sulfate—copper sulfate mixture*.—Thoroughly mix 16 parts of  $K_2SO_4$  with 1 part of  $CuSO_4 \cdot 5H_2O$ .

(i) *Wash soln*.—Mix 980 cc of  $H_2O$  with 20 cc of  $HNO_3$ .

## 39

## DETERMINATION

Weigh into a beaker such a quantity of the sample as is expected to contain 10–40 mg of Cl. (If more than 4 g is taken, use proportionately more  $HNO_3$  and  $KMnO_4$  soln.) Add 10 cc of the 0.3 N  $AgNO_3$  soln and stir until the sample is thoroughly soaked with the soln, adding a little  $H_2O$  or warming if necessary. Add 25 cc of  $HNO_3$ , stir, add 5 cc of the  $KMnO_4$  soln, and stir until the frothing stops. Place the mixture in a water bath or on a hot plate to keep it just below boiling. Stir, and wash down the sides of the beaker at intervals with the least possible quantity of  $H_2O$ . After 20 min., or when there appears to be no further action on the sample, add more of the  $KMnO_4$  soln, a little at a time until the color fades slowly or until twice the amount used at the beginning has been added. Dilute to about 125 cc with boiling  $H_2O$  and heat 10 min. longer. (The beaker may stand in the bath or on the hot plate until ready to filter.) Filter while hot thru Whatman's No. 5, or similar paper, with suction as follows: Place a disk of 30-mesh stainless steel wire gauze or of No. 40 filter cloth in the bottom of a 3 in. Hirsch funnel. Fold a 9 cm paper over the bottom of a No. 11 rubber stopper, shaping it to the funnel by making 9–10 folds up the side of the stopper. Place the paper in the funnel and apply strong suction. Wet the paper and keep it wet while fitting it into the funnel so as to avoid double thicknesses of paper. Thoroughly wash the paper, first with  $H_2O$  then with the wash soln. Discard the washings and rinse out the flask. Pour the supernatant liquid thru the filter and transfer the precipitate and sample residue to the filter. If the filtrate is not turbid or only slightly opalescent, wash the precipitate thoroughly, applying the wash soln very gently, but maintaining a strong suction on the filter. If the combined filtrate and washings are clear, test them for Ag. If turbid, re-heat and pass thru the filter, repeating until clear, and finally wash as directed above. If the filtrate does not give a definite test for Ag, repeat the determination on a fresh but smaller portion of the sample. Place the filter paper and contents in a Kjeldahl flask and add such quantities of the mixture of  $CuSO_4$  and  $K_2SO_4$  and of  $H_2SO_4$  as would be appropriate for a protein determination on the same kind and amount of material and digest in a similar manner. (For 2 g of grass, 8 g of the sulfate mixture and 20 cc of acid are enough.) When the digest is cool, add 75 cc of  $H_2O$  and cool to room temp. Titrate the  $Ag_2SO_4$  in the Kjeldahl flask with the standard KI, using

5 cc of starch and 30 cc of I as indicator. (The latter is added just before the titration.) Wash down the neck of the flask after each addition of KI when near the end point and titrate until the blue color persists after shaking. If less than 30 mg of chlorine is present, add the starch and I at the beginning. If a larger but unknown amount is present, add 2 cc of starch and 10 cc of I at the beginning and titrate until the approach of the end point is seen. Shake vigorously to coagulate the precipitate, add the remainder of the starch and I and proceed to the end point. If a known large amount is present, titrate to within 2 cc of the end point, shake as above, add the indicator reagents, and continue the titration. If the end point is over-run, add 5 cc of the standard  $\text{AgNO}_3$  and titrate again.

Blank determinations are not necessary after the reagents have been tested. If the blanks made by using pure sugar as a sample exceed 0.05 mg, examine the filter paper and the various reagents carefully.

## 40

IODINE<sup>27</sup>—TENTATIVE

Weigh a sample of 50 g of finely ground, air-dry plant material; transfer to a porcelain dish, and thoroly mix with 10 g of finely pulverized  $\text{CaO}$  and 10 g of finely pulverized  $\text{CuO}$ . Distribute the mixture in 3 alundum boats and place end to end in a large combustion tube. Close the right end of the combustion tube tightly with a rubber stopper that carries a glass tube connecting with the wash bottle. Connect the wash bottle on the left to a suction pump, and close the electric circuit. When tube 3 attains a red heat draw air thru the system and light the first burner on the left of the gas furnace. (After a short time the heat from this burner sets the plant material in the first boat on fire, and a moderately rapid current of air drawn thru the tubes and heat from the other burners of the gas furnace lighted at the proper time keep the sample burning at a slow and uniform rate, somewhat in the manner of a lighted cigar. Any unburned vapors from the sample are drawn over the red-hot platinized asbestos catalyst, where they are completely burned, and the I vapors are carried into the gas wash bottles and absorbed.)

After the combustion is completed, turn off the heat and cool the apparatus by continuing to draw the current of air thru the system. Disconnect the suction pump and remove the boats carefully. Digest the ash, leach with hot distilled  $\text{H}_2\text{O}$ , and combine the filtrate from the ash with the  $\text{K}_2\text{CO}_3$  solns from the absorption flasks and evaporate to dryness.

Add just enough  $\text{H}_2\text{O}$  to dissolve the residue and transfer the soln to a separator of the proper size. Add enough 95% pure ethyl alcohol to form two immiscible layers and shake the separator vigorously for about 10 min. Run the aqueous portion of the soln into another separator, and repeat the process of extraction 3 times. Combine the alcoholic extracts that contain the I and evaporate slowly in a small silica dish to dryness, avoiding spattering. Heat in an electric furnace having a pyrometer attachment for 30 min. at  $400^\circ$  to char any organic matter present.

Cool, dissolve the residue in a few drops of  $\text{H}_2\text{O}$ , filter into a small separator, and make slightly acid with  $\text{H}_2\text{SO}_4$  (1+3). Add about 3 cc of a saturated soln of sulfuric acid; stopper the separator and shake vigorously for about 1 min. to reduce iodate, if present, to iodide. Add 1 cc of pure  $\text{CS}_2$ , accurately measured, and about 2 cc of a 10% soln of I-free  $\text{NaNO}_2$ . Stopper the funnel and shake vigorously for about 1 min. and allow the  $\text{CS}_2$  to settle. (If in the  $\text{CS}_2$  a slight pink color is evident, all the I has been absorbed.) If the  $\text{CS}_2$  has a deep pink color, run it into a centrifuge tube, add 1 cc of  $\text{CS}_2$ , and repeat the extraction until the last portion has only a faint color. Combine the extracts, centrifuge, and compare a portion in a micro-

colorimeter with an I standard prepared in a similar way. Report the results in p.p.m. if high, or p.p.b. if low.

1. Absorption bottle containing 5%  $K_2CO_3$  (2 bottles used); 2. rheostat; 3. silica catalyst tube; 4. platinized asbestos catalyst; 5. electric tube furnace, maximum temp.  $1100^\circ$ ; 6. asbestos cement seal, sealing large combustion tube to smaller tube containing catalyst; 7. silica combustion tube; 8. alundum boats containing sample; 9. gas combustion furnace; and 10. wash bottle containing 10%  $K_2CO_3$ .

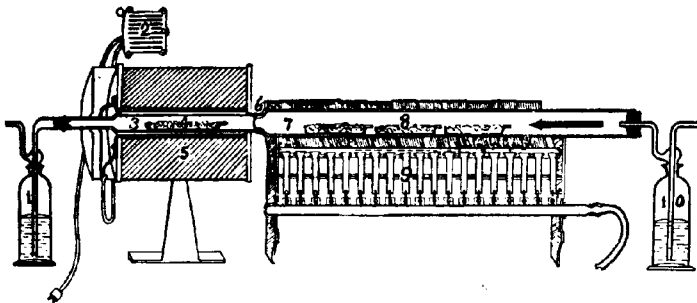


FIG. 15.—APPARATUS FOR DETERMINATION OF IODINE

## 41

SELENIUM<sup>13</sup>

(Applicable to materials containing over 2 p.p.m. of selenium.)

Grind the air-dried sample and carefully prepare a uniform subsample. Prepare a mixture of 50 cc of  $H_2SO_4$  and 100 cc of 68%  $HNO_3$  in a 600 cc beaker. Add 5 g of the powdered sample to the acid mixture slowly, with stirring, restricting the temp. of the mixture to not above  $80^\circ$ . After adding the sample and the first vigorous reaction is over, warm gently with occasional stirring until fumes of nitrogen peroxide cease to evolve. Warm at a temp. not to exceed  $120^\circ$  until a slight darkening of the liquid begins to appear. Transfer the liquid to a distilling apparatus and proceed as directed in I, 33.

SUGARS<sup>14</sup>

## 42

## PREPARATION OF SOLUTION—OFFICIAL, FIRST ACTION

(a) *Extraction*.—Prepare the sample as described under 2(b). Pour the alcoholic soln thru a filter paper or extraction thimble, catching the filtrate in a volumetric flask. Transfer the insoluble material to a beaker, cover with 80% alcohol, warm on a steam bath for 1 hour, allow to cool, and again pour the alcoholic soln thru the same filter. If the second filtrate is highly colored, repeat the extraction. Transfer the residue to the filter, allow to drain, and dry. Grind the residue so that all the particles will pass thru a 1-mm sieve, then transfer it to an extraction thimble and extract for 12 hours in a Soxhlet apparatus with 80% alcohol. Dry the residue and save for the starch determination. Combine the alcoholic filtrates and make to volume at a definite temp. with 80% alcohol.

(b) *Clearing*.—Place an aliquot of the alcoholic extract in a beaker on the steam bath and drive off the alcohol. Avoid evaporation to dryness by adding  $H_2O$  if necessary. When the odor of alcohol has disappeared from the sample, add about 100 cc of distilled  $H_2O$  and heat to  $80^\circ$  to soften gummy precipitates and break up insoluble masses. Cool to room temp. and proceed as directed under (1) or (2).

(1) Transfer the soln to a volumetric flask and rinse the beaker thoroly with

H<sub>2</sub>O, adding the rinsings to the contents of the flask. Add enough saturated neutral Pb acetate to produce a flocculent precipitate, shake thoroly, and allow to stand 15 min. Test the supernatant liquid with a few drops of saturated Pb acetate. If more precipitate forms, shake and allow to stand again; if no further precipitate forms, dilute to the mark with H<sub>2</sub>O, mix thoroly, and filter thru a dry filter. Add sufficient solid Na oxalate to the filtrate to precipitate all the Pb, and refilter thru a dry paper. Test the filtrate for the presence of Pb with a little solid Na oxalate.

(2) Add double the minimum amount of saturated neutral Pb acetate soln that is required to cause complete precipitation, as found by testing a portion of the supernatant liquid with a few drops of dilute Na oxalate soln. After allowing the mixture to stand a few min. only, filter immediately into a beaker to which has been added an estimated excess of Na oxalate crystals. Allow the Pb precipitate to drain on the filter and wash with cold H<sub>2</sub>O until the filtrate no longer gives a precipitate in the oxalate soln. Excess of oxalate must be assured by testing with a drop of dilute Pb acetate soln. Filter off and wash the precipitated Pb oxalate, catching the filtrate and washings in a volumetric flask. Dilute to the mark with H<sub>2</sub>O and mix.

#### REDUCING SUGARS

##### 43 *Munson and Walker General Method—Tentative*

Proceed as directed under XXXIV, 36.

*Quisumbing and Thomas Method<sup>20</sup>—Official, First Action*

##### 44

#### REAGENTS

(a) *Copper sulfate soln.*—Wash crystals of C.P. CuSO<sub>4</sub>·5H<sub>2</sub>O free from dust, etc., with H<sub>2</sub>O, dissolve in hot H<sub>2</sub>O to make a saturated soln, and filter. Determine the Cu electrolytically and dilute the soln so that 25 cc of it will contain 525 mg of Cu or 41.2 g of CuSO<sub>4</sub>·5H<sub>2</sub>O in 500 cc of soln.

(b) *Alkaline tartrate soln.*—Prepare a saturated soln of NaOH (purified by alcohol) and let stand until the insoluble carbonates and other impurities have settled out several days. Siphon off the clear soln and establish its alkalinity by titration with standard acid. Dissolve 173 g of highest purity Rochelle salts in H<sub>2</sub>O in a 500 cc graduated flask and add the calculated quantity of NaOH soln so that 500 cc of this alkaline tartrate soln will contain exactly 65 g of NaOH. Make to the mark with H<sub>2</sub>O.

##### 45

#### DETERMINATION

Measure exactly 25 cc each of the CuSO<sub>4</sub> and alkaline tartrate solns into a 400 cc Pyrex or Bohemian glass beaker, the diameter of which is about 9 cm. Add 50 cc of sugar soln containing preferably 50–150 mg of sugar. Cover the beaker with a watch-glass and place the beaker in a water bath which is maintained at 80°. After digesting exactly 30 min., filter the Cu<sub>2</sub>O by suction thru a mat of asbestos in a Gooch crucible. Wash the precipitate with H<sub>2</sub>O. Determine the Cu by one of the methods below. Calculate the weight of sugar from the tables of Quisumbing and Thomas (XLII, 17).

##### 46

(a) *Direct Weighing of Cuprous Oxide—Official, First Action*

Proceed as directed under XXXIV, 39.

##### 47

(b) *Volumetric Permanganate Method<sup>21</sup>—Tentative*

Filter and wash the Cu<sub>2</sub>O as directed under 45. With the aid of a stirring rod transfer the asbestos mat and the Cu<sub>2</sub>O back into the beaker in which the reduction

took place.\* Rinse the inside of the crucible and the lip of the beaker with 10 cc of a soln of 240:9 g of crystalline ferric  $(\text{NH}_4)_2\text{SO}_4$  and 200 cc of  $\text{H}_2\text{SO}_4$  dissolved in  $\text{H}_2\text{O}$  and made up to 1 liter. Cool the diluted  $\text{H}_2\text{SO}_4$  before adding the salt. Receive the rinsings in the beaker containing the  $\text{Cu}_2\text{O}$ . Holding the crucible over the beaker, stir the contents of the beaker thoroly with the stirring rod until the  $\text{Cu}_2\text{O}$  has gone into soln. Wash the crucible with about 25 cc of hot  $\text{H}_2\text{O}$  ( $80^\circ$ ), receiving the washings in the beaker. Stir the contents of the beaker and then raise the beaker to see if any undissolved particles of  $\text{Cu}_2\text{O}$  are resting on the bottom. If any undissolved particles are present, press out each one with the point of the stirring rod until all have gone into soln. Add about 125 cc more of hot  $\text{H}_2\text{O}$ . Add 1 drop of a soln of 0.15 g of orthophenanthroline monohydrate and 0.07 g of ferrous sulfate in 10 cc of  $\text{H}_2\text{O}$ . Titrate at once with continual stirring with 0.05 N  $\text{KMnO}_4$ . (In a long titration it is best to add the indicator just before the end point is reached.) Standardize the  $\text{KMnO}_4$  as follows: Dry overnight about 0.5 g of Na oxalate (U. S. Bureau of Standards) in an oven at  $100^\circ$  and carefully weigh out into beakers 3 samples of about 0.10–0.15 g each. Dissolve each sample in about 100 cc of  $\text{H}_2\text{O}$ , add 5 cc of  $\text{H}_2\text{SO}_4$  (1+1), warm to  $70^\circ$  and titrate the  $\text{KMnO}_4$  against this soln, stirring the liquid vigorously and continuously. Subtract from the titration the excess  $\text{KMnO}_4$  needed to obtain the end point color as determined by matching the color in another beaker containing the same bulk of acid and hot  $\text{H}_2\text{O}$ . The temp. of the soln should not be below  $60^\circ$  by the time the end point is reached. 1 cc of 0.05 N  $\text{KMnO}_4 = 0.00335$  g of Na oxalate and 0.0031785 g of Cu, or

$$\text{mg of Cu per cc of KMnO}_4 = \frac{\text{g of Na oxalate} \times 948.8}{\text{cc of KMnO}_4}$$

48 (c) *Electrolytic Deposition from Sulfuric and Nitric Acid Solutions—Tentative*

Proceed as directed under XXXIV, 42.

49 SUCROSE—TENTATIVE

(a) *Hydrochloric acid inversion*

Proceed as directed under XXVII, 30.

(b) *Invertase Inversion*

When glucosides which are easily hydrolyzed by HCl are present, sucrose may be inverted by invertase as directed under XXXIV, 21. The quantity of invertase to be used depends on its activity, but a large excess should be avoided because it causes difficulty in the filtration of the reduced Cu.

STARCH—TENTATIVE

50 *Diastase Method with Subsequent Acid Hydrolysis*

Proceed as directed under XXVII, 33. If the sample has been previously extracted in a Soxhlet with hot alcohol, further extraction with alcohol and ether is unnecessary.

51 ETHER EXTRACT—TENTATIVE

Proceed as directed under XXVII, 22.

52 CRUDE FIBER—TENTATIVE

Proceed as directed under XXVII, 27.

53

## TOTAL NITROGEN

Proceed as directed under II, 27.

AMMONIA IN TOBACCO<sup>22</sup>—TENTATIVE

54

## REAGENTS

(a) *Ammonium sulfate stock soln.*—Dissolve 2.358 g of pure salt in H<sub>2</sub>O and make up to 1000 cc. 2 cc = 1.0 mg of N. Preserve by adding a few drops of CHCl<sub>3</sub>.

(b) *Ammonium sulfate standard soln.*—Dilute 200 cc of (a) to 1000 cc; 1 cc = 0.1 mg of nitrogen. Preserve with CHCl<sub>3</sub>.

(c) *Nessler's soln* (Folin\*).—Transfer 37.5 g of KI and 27.5 g of I to a 250 cc flask, and add 25.0 cc of H<sub>2</sub>O and 35–40 g of Hg. Shake the flask continuously and vigorously for 7–15 min., or until nearly all the dissolved I has disappeared. (The soln becomes hot.) When the red I soln has begun to pale visibly, though still red, cool in running H<sub>2</sub>O, and continue the shaking until the reddish color of the I has been replaced by the greenish color of the double iodide. (This whole operation should not take more than 15 min.) Separate the soln from the surplus Hg by decantation and washing with liberal quantities of H<sub>2</sub>O. Dilute the soln and washings to 500 cc. If the cooling was begun in time, the resulting concentrated soln of the double iodide is clear enough for immediate dilution with 10% NaOH and H<sub>2</sub>O and the finished Nessler's reagent can be used at once. Place 700 cc of 10% NaOH soln in a 1 liter flask, add 150 cc of the clear concentrated soln of the double iodide, mix, and dilute to 1 liter with H<sub>2</sub>O. Allow to settle if a turbidity develops.

(d) *Permutit* (Folin\*).—Pass through sieves and reject material smaller than 80-mesh and larger than 60-mesh. Wash copiously with H<sub>2</sub>O by decantation until the whole settles rapidly and contributes no more dust or turbidity to the H<sub>2</sub>O. Dry in a current of air in a thin layer without heating. For recovery of permutit after use, see Folin's manual.\*

55

## DETERMINATION

Transfer an accurately weighed 0.5 g sample of dry finely powdered tobacco to a 300 cc Kjeldahl flask; add 25–30 cc of H<sub>2</sub>O, a small piece of paraffin, a few angular quartz pebbles, and 2–2.5 g of light MgO. Prepare a stopper to fit the Kjeldahl flask with a piece of 9 mm outside diameter glass tubing bent around thru 180°, the short limb of the bend inserted thru the stopper and the longer limb reaching to the level of the desk as the flask is held in a clamp over a micro burner. (Greater convenience is obtained if the longer limb is cut and joined again by a short length of rubber tubing at a point about 15 cm from the lower end.) Connect the distillation tube so prepared to the flask and dip the lower end into a short wide test tube (50 cc centrifuge tube) that contains 5 cc of 0.1 N HCl and a few drops of methyl red indicator, II, 19(i). Heat the contents of the flask with a micro burner at such a rate that steam begins to rise from the receiver in about 3 min. Make no effort to cool the distillation tube or receiver. Distil for 5 min., counting the time from the point at which the distillate first runs down the tube; remove the tube and wash the end into the receiver with a few drops of H<sub>2</sub>O; cool the distillate and dilute to 50 cc. Charge several 100 cc volumetric flasks with 2.5–3.0 g of the washed and dried permutit and wash each several times by decantation with H<sub>2</sub>O. Transfer to three of the flasks 3 cc of 0.1 N HCl and portions of the standard (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> soln (b) containing 0.3, 0.5, and 1.0 mg of N, respectively. Add sufficient H<sub>2</sub>O to each flask

\* Laboratory Manual of Biological Chemistry, New York, 4th ed., p. 293 (1926). The reagent prepared by the usual procedure is, however, equally satisfactory.

to make a total volume of 25 cc. Transfer a 25 cc aliquot of the distillate from each determination to a flask containing permittit. Shake all the flasks for 5 min. with a gentle rotatory motion and lay them on their sides on a suitable support for 1 min.; decant the fluid from each flask and wash the permittit by decantation 3 times successively with 10–30 cc of  $H_2O$ , allowing the soln to settle for 1 min. before each decantation. Rinse the permittit to the bottom of each flask with 5 cc of  $H_2O$ , add 1 cc of 10% NaOH soln, and rotate for 3 min.; add 65 cc of  $H_2O$ , rotate, and add 10 cc of the Nessler reagent. Dilute to the mark, mix, and compare in a colorimeter the color of the soln derived from each determination with the known standard that most nearly matches it. (The color is stable for several hours.) Calculate the ammonia nitrogen as percentage of the sample of tobacco used.

#### FREE NICOTINE IN TOBACCO<sup>12</sup>—TENTATIVE

56

##### DETERMINATION

Mix approximately 2.5 g of dry powdered tobacco with 50 cc of  $H_2O$ . Stir for 5–10 min., allow to settle, and decant the necessary quantity into the cell of a quinhydrone or hydrogen electrode. Determine the pH value with an accuracy of 0.1 unit. Construct a curve by plotting the data in the following table on a conveniently large scale. Read the percentage of free nicotine from this curve at a point corresponding to the pH found and report as percentage of the total nicotine in the free form.

57	FREE NICOTINE per cent	pH	FREE NICOTINE per cent	pH
	1	6.11	50	8.11
	2	6.42	55	8.20
	5	6.86	60	8.29
	10	7.15	65	8.37
	15	7.36	70	8.48
	20	7.51	75	8.59
	25	7.63	80	8.71
	30	7.74	85	8.86
	35	7.85	90	9.06
	40	7.93	95	9.39
	45	8.02		

#### NITRATE NITROGEN<sup>13</sup>—TENTATIVE

(Applicable to tobacco and other plant tissues.)

58

##### REAGENTS

- (a) *Sulfuric acid soln.*—4 N. Prepared from C.P. special reagent low in N.
- (b) *Sulfuric acid soln.*—18 N. Prepared from C.P. special reagent low in N.
- (c) *Reduced iron powder.*—Determine the titration value of the ammonia in the powder by boiling 3.0 g of it with 50 cc of 4 N  $H_2SO_4$  for 5 min., cooling, making alkaline with NaOH, distilling into 0.1 N acid, and titrating with 0.1 N alkali to methyl red. Divide by 10 to obtain the correction to be used with the 0.3 g of powder used in the method.
- (d) *Ammonium sulfate stock soln.*—Dissolve 2.358 g of pure salt in  $H_2O$  and make up to 1000 cc; 2 cc = 1.0 mg of N. Add no preservative.
- (e) *Ammonium sulfate standard soln.*—Dilute 200 cc of (d) to 1000 cc; 1 cc = 0.1 mg of N.
- (f) *Diphenylamine soln.*—Suspend 0.5 g of diphenylamine in 20 cc of  $H_2O$  and add  $H_2SO_4$  to make 100 cc. Cool and preserve in a dark bottle.

Ascertain the quantity of 4  $N$   $H_2SO_4$  required to bring a 2 g sample of the dried and powdered tissue to approximately pH 1.0 as follows: Weigh out 0.5 g, stir in a small beaker with 1 cc of 4  $N$  acid, add enough  $H_2O$  to make a thin paste that can be transferred to the electrode vessel, add quinhydrone, and determine the reaction at the potentiometer. Make suitable changes in the quantity of acid added to a second 0.5 g sample, as suggested by the result of the first test, and repeat the determination. Continue until the quantity required to give a reaction in the range pH 0.7–0.9 has been found. Multiply this quantity by 4 to obtain the amount required by the 2 g sample used for the nitrate determination.

Weigh duplicate 2 g samples of the powder, mix each in a beaker with the required quantity of 4  $N$   $H_2SO_4$  until a uniform stiff paste is obtained; add 3.5 g of pure asbestos fiber to each and incorporate thoroly. Transfer the mixtures to 26×60 mm paper extraction thimbles by means of a glass funnel about 11 cm long, the upper part of which is a cylinder 4.5 cm in diameter, the lower a cylinder 2 cm in diameter. The transfer is accomplished as follows: Support the thimble in a wire cage hung in the mouth of a 400 cc conical extraction flask and clamp the funnel in position over it so that the smaller end extends about 1 cm into the thimble. Push most of the asbestos mixture into the thimble with a glass rod; brush off beaker, funnel, and rod; and wipe off all particles with a small piece of surgical cotton. Finally rinse the glassware and brush into the thimble with alcohol-free ether. Remove the funnel and plug the end of the thimble with the cotton used to wipe the apparatus. Place the thimble in the siphon tube of the ether extraction apparatus (type designed for rubber analysis), thrust a short slim glass rod between the thimble and the glass in order to hold the paper away from the glass wall at one side, and suspend the siphon tube close under the metal coil condenser of the apparatus by means of a fine galvanized iron wire. Place 150–200 cc of alcohol-free ether in the conical flask. Cut a gasket from soft cardboard to fit the recess in the plate of the metal condenser and set the condenser, with attached siphon tube, on the extraction flask and hold it firmly in position by means of three spring paper clips.

Place the extraction flasks on an electric hot plate, add a few angular quartz pebbles, and allow the extraction to proceed at least 8 hours at a siphoning rate of about 40 times per hour. (If the rate is less a correspondingly longer time must be allowed.) To test for the completeness of the removal of the  $HNO_3$  prepare a concentrated water extract of the residue in the thimble and overlay 5 cc of the diphenylamine reagent in a test tube with a few cc of this extract. (The appearance of a blue layer at the junction of the two solns indicates that the extraction of the  $HNO_3$  by the ether has been incomplete.)

Treat each ether extract with 25 cc of  $H_2O$ , add 2 drops of phenolphthalein, and make faintly alkaline with 0.5  $N$   $NaOH$  with continual agitation. Immerse the flask in a water bath and evaporate off the ether very slowly to avoid frothing; make the aqueous soln to 100 cc and transfer an aliquot (10 cc or more, depending on the nitrate content of the tissue) to a 300 cc Kjeldahl flask. Add 2.5 cc of the 18  $N$   $H_2SO_4$  and 0.3 g of the reduced iron powder. Boil gently for 5 min., cool, add 20 cc of  $H_2O$  and 10 cc of ammonia-free concentrated  $NaOH$ . Immediately fit the flask with a Folin and Wright distillation tube and distil as directed under 55, into 3 cc of 0.1  $N$   $HCl$  contained in a test tube. Transfer the distillate to a 100 cc flask, dilute to about 60 cc with ammonia-free  $H_2O$ , add 10 cc of the Nessler's soln, agitate, and make to volume. Prepare standard ammonia solns by pipetting 3–15 cc of the  $(NH_4)_2SO_4$  standard soln into 100 cc flasks (0.3–1.5 mg ammonia nitrogen), dilute,



add 10 cc of Nessler's soln, agitate, and make to volume. Read the color of the soln derived from the analysis in a colorimeter against nearest standard. Calculate the quantity of N in the aliquot used for reduction, subtract the blank for the ammonia nitrogen found in the 0.3 g of iron powder used, and calculate the nitrate nitrogen in the 2.00 g sample taken. Express the final result as percentage of the dry tissue.

If the nitrate content of the tissue is 0.1% or less, it is desirable to carry out a blank determination on the alkaline soln of the ether extract. To do this proceed as follows:

Transfer an aliquot of the alkaline soln equal to that used for the determination to a 300 cc Kjeldahl flask, add 2.5 cc of the 18 N  $\text{H}_2\text{SO}_4$ , boil gently for 5 min., cool, add 20 cc of  $\text{H}_2\text{O}$  and 10 cc of 0.5 N NaOH soln, and distil as already described. Transfer the distillate to a 25 cc volumetric flask, dilute to 15 cc, add 2.5 cc of Nessler's soln, agitate, and make to volume. Compare with ammonia standards of 0.05–0.10 mg. Deduct the quantity of ammonia nitrogen found from the quantity found after reduction with the iron powder, correct the result for the blank due to the iron powder, and calculate the nitrate nitrogen as before.

To determine the nitrate content of extracts from plant tissue proceed as follows:

Transfer an aliquot of the extract approximately equivalent to 2 g of dry tissue to an evaporating dish, make neutral to Congo red if necessary, and evaporate to a sirup (it must not be evaporated to dryness). Cool, and add the quantity of the 4 N  $\text{H}_2\text{SO}_4$  found by a separate experiment to be required to produce a reaction in the range pH 0.7–0.9; add 3.5 g of asbestos, and mix thoroly. If the mixture is too moist to be transferred to the extraction thimble, dry it in a vacuum desiccator until this can be done. Proceed with the extraction as already described.

#### LIGNIN<sup>24</sup>—TENTATIVE

##### 60

##### PREPARATION OF SAMPLE

Grind the plant material in a mill to pass thru an 80-mesh sieve, and dry at 105°. Extract a weighed sample (5–10 g) for 30 hours in a Soxhlet apparatus with an alcohol-benzene soln (32 parts by weight of 95% ethanol and 68 parts by weight of benzene). Dry the material in an oven to free it from the alcohol-benzene soln and place in a flask of suitable size. Add  $\text{H}_2\text{O}$  in the proportion of 150 cc to 1 g of sample, and boil the mixture under a reflux condenser for 3 hours. Filter the mixture while still hot, preferably thru a weighed sintered-glass crucible, and transfer the extracted material to a flask. Add a 1% HCl soln in the proportion of 150 cc of acid soln to 1 g of plant material, and boil under a reflux condenser for 3 hours. Filter the mixture while still hot thru the sintered-glass crucible used in the previous operation, wash with  $\text{H}_2\text{O}$  until free of acid, dry at 105°, and weigh. Calculate the percentage total loss due to the successive extraction with the alcohol-benzene soln, hot  $\text{H}_2\text{O}$ , and the 1% HCl. (In substances not especially rich in carbohydrates and proteins, the extraction with hot  $\text{H}_2\text{O}$  may be omitted.)

##### 61

##### APPARATUS

The apparatus required is illustrated in Fig. 16. It consists of a bottle (A) having a capacity of 1500 cc and containing approximately 500 cc of  $\text{H}_2\text{SO}_4$ . Attached to A by means of a two-holed rubber stopper is a 250 cc dropping funnel (C), having the lower end of its stem bent as illustrated, containing HCl. By means of stopcock B, allow the HCl to flow into the  $\text{H}_2\text{SO}_4$ , and dry the HCl gas thus generated by passage thru the  $\text{H}_2\text{SO}_4$  in D. The lower end of the stem of C must be close to the

bottom of A, as shown in the drawing. Place the weighed sample and the fuming HCl in a Pyrex test tube 300 mm long and 38 mm in diameter (G). By means of the device O, connect G in parallel to two other tubes, G' and G'' (see top view), having the same dimensions as G, and provide G, G', and G'' with two-holed rubber stoppers. Thru one hole pass a glass tube having a right-angled bend nearly to the bottom of the large test tube (F, F', and F''). Thru the other hole insert another tube having a right-angled bend, which extends about 10 mm into the large test tube. K is a bottle containing H<sub>2</sub>O for the absorption of the excess HCl gas that passes thru device P and tube J. Regulate the flow of HCl gas thru the three large test tubes by means of the stopcocks shown in the top view. Place

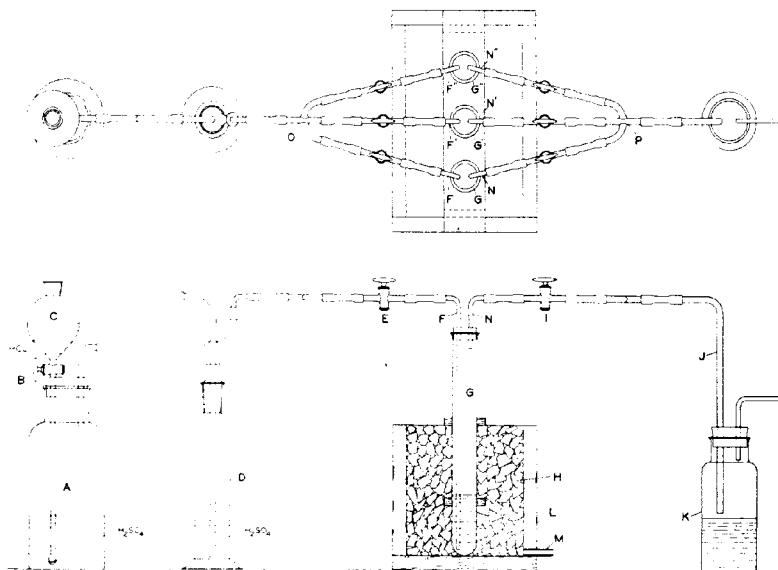


FIG. 16.—APPARATUS FOR DETERMINATION OF LIGNIN

G, G', and G'' in the wooden box L, provided with supports for the tubes and also with a drain (M) for the removal of the H<sub>2</sub>O. Surround the large test tubes G, G', and G'' in L with crushed ice (H).

## 62

## REAGENT

*Fuming hydrochloric acid.*—Density 1.212–1.223 at 15°. Prepare as follows: To 500 g of NaCl contained in a liter Pyrex distilling flask provided with a ground-glass stopper, add a cold soln of 250 cc of H<sub>2</sub>O in 450 cc of H<sub>2</sub>SO<sub>4</sub>. Connect the side tube of the distilling flask to a glass tube which passes thru a H<sub>2</sub>SO<sub>4</sub> wash bottle. Connect the outlet tube of the H<sub>2</sub>SO<sub>4</sub> wash bottle to another glass tube which is immersed in a flask containing 3 liters of HCl. Surround the flask containing the HCl with crushed ice. Heat the distilling flask with a small flame and pass the HCl gas into the acid soln until it attains the sp. gr. 1.212–1.223 at 15°. Keep the reagent in a refrigerating room maintained at a temp. of 0° or below. If only a few determinations are to be made, a correspondingly smaller quantity should be prepared.

## 63

## DETERMINATION

Weigh out three 1 g samples of the extracted and dried material in a weighing bottle and place in the three large test tubes, G, G', and G''. Add 20 cc of the reagent to each tube, taking care to wash down with this acid any particles clinging to the sides. When all the material is wetted with the reagent, add another portion (30 cc). Add about 3 drops of capryl alcohol to reduce the foaming to a minimum during the subsequent passage of the HCl gas thru the reaction mixture. Place the three large test tubes, G, G', and G'' in the wooden box (L) and surround with crushed ice. Lubricate tubes F, F', and F'' with a drop of glycerol so that they move easily thru the holes in the rubber stoppers. Then lead the dry HCl gas from the generator into the reaction mixtures thru the tubes F, F', and F'' (F' and F'' are shown in top view), which reach nearly to the bottom of the tubes G, G', and G''. Regulate the flow of the gas thru the reaction mixtures in G, G', and G'' by means of the stopcocks shown in the top view, continuing the passage of the gas for 2 hours. (At first a rather slow stream of gas passes in, but during the last 15 min. the flow is fairly rapid.) At the end of the reaction period discontinue the flow of the gas, and disconnect the long tubes F, F', and F'' and the outlet tubes of the three test tubes G, G', and G'' from O and P. (The tubes F, F', and F'' are pulled up just above the surface of the reaction mixture and are closed by means of short pieces of rubber tubing having one end plugged with a short piece of glass rod.) Similarly close off the outlet tube N and the outlet tubes of G' and G''. Place the tubes containing the reaction mixture in a cold room or ice box (temp.  $+8^{\circ}$ – $+10^{\circ}$ ) and allow to remain there for 24 hours. Transfer the contents of the tubes G, G', and G'' to 1 liter Erlenmeyer flasks, taking care to remove any material adhering either on the inside or outside of the tubes F, F', and F''. Dilute the reaction mixtures with  $H_2O$  to a volume of 500 cc. Connect the flasks to reflux condensers and boil for 1 hour. Prepare three Gooch crucibles in the usual manner, dry at  $105^{\circ}$ , and weigh. Ignite one of the weighed crucibles, A, on a Bunsen burner, cool in a desiccator, and reweigh. Allow the contents of the flasks to cool to room temp. and filter thru weighed Gooch crucibles. Wash the precipitates collected in the Gooch crucibles with hot  $H_2O$ , dry at  $105^{\circ}$ , and weigh in a weighing bottle. Ignite the crude lignin in crucible A over a Bunsen flame and determine the weight of ash. Place one of the other two Gooch crucibles in a Kjeldahl flask provided with a wide neck, and determine the percentage of nitrogen in the crude lignin as directed in II, 25. If it is desired to determine the percentage of methoxyl in the lignin, collect the precipitate from one of the flasks in a dried ( $105^{\circ}$ ) sintered glass crucible. Compute the weight of lignin in the sample as follows: Weight of lignin = weight of crude lignin – weight of ash – weight of crude protein ( $N \times 6.25$ ). Calculate the percentage of lignin in the original dry unextracted material.

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- <sup>19</sup> Ibid., **14**, 73, 225 (1931); **15**, 71 (1932).
- <sup>20</sup> J. Am. Chem. Soc., **43**, 1503 (1921); J. Assoc. Official Agr. Chem., **15**, 71 (1932).
- <sup>21</sup> J. Assoc. Official Agr. Chem., **19**, 72 (1936).
- <sup>22</sup> Ibid., **14**, 229 (1931); **16**, 71 (1933).
- <sup>23</sup> Ibid., **16**, 474 (1933); **17**, 63 (1934).
- <sup>24</sup> Ibid., **15**, 124 (1932); **18**, 386 (1935); **19**, 107 (1936).

### XIII. BEVERAGES (NON-ALCOHOLIC) AND CONCENTRATES

#### 1 PRELIMINARY EXAMINATION

Note and record (a) appearance, whether bright or turbid, or any sediment; (b) color and depth of color; (c) odor, whether fruity, foreign, or artificial; (d) taste, whether tart or sweet, fruity, artificial or foreign, and whether any synthetic substance can be identified by odor or taste.

#### 2 SPECIFIC GRAVITY—OFFICIAL

Proceed as directed under XIV, 3.

#### 3 ALCOHOL—OFFICIAL

Proceed as directed under XIV, 5.

#### 4 TOTAL ACIDITY—OFFICIAL

Proceed as directed under XVI, 41.

### CHARACTERISTIC ACIDS

#### 5 PRELIMINARY PROCEDURE

(a) *Alcoholic products*.—Proceed as directed under XVI, 42.

(b) *Non-alcoholic products*.—Measure out a volume of sample that contains not more than 30 g of solid matter and not more than 200 mg of the acid to be determined as calculated from the acidity. Evaporate to 30 cc if necessary, add 3 cc of 1 N H<sub>2</sub>SO<sub>4</sub>, and transfer to a 250 cc volumetric flask, using 10 cc of H<sub>2</sub>O and sufficient 95% alcohol to fill the flask to the mark. Mix, and allow to stand 15 min. Filter thru a thin layer of absorbent cotton, protecting the liquid against evaporation. Transfer 200 cc of the filtrate to a centrifuge bottle and proceed with the determination of the acid as directed below.

#### 6 TARTARIC ACID—TENTATIVE

Using the material in the centrifuge bottle, proceed as directed under XXVI, 26.

#### 7 CITRIC ACID—TENTATIVE

Using the material in the centrifuge bottle, proceed as directed under XXVI, 31.

#### 8 MALIC ACID—TENTATIVE

Using the material in the centrifuge bottle, proceed as directed under XXVI, 32.

#### 9 VOLATILE ACIDS—OFFICIAL

Proceed as directed under XV, 23.

#### 10 ESTERS—OFFICIAL

Proceed as directed under XVI, 9.

#### 11 TOTAL SOLIDS—OFFICIAL

Proceed as directed under XXXIV, 3 or 4.

#### SUCROSE

#### 12 By Polarization—Official

Determine by polarizing before and after inversion as directed under XXXIV, 22, 23 or 25.

13 *By Reducing Sugars Before and After Inversion—Official*

Proceed as directed under XXXIV, 28.

14 REDUCING SUGARS—OFFICIAL

Proceed as directed under XXXIV, 37, and express the results as invert sugar.

15 COMMERCIAL GLUCOSE—OFFICIAL

Proceed as directed under XXXIV, 30.

16 ASH—OFFICIAL

Proceed as directed under XXXIV, 8 or 9.

17 SOLUBLE AND INSOLUBLE ASH—OFFICIAL

Proceed as directed under XXXIV, 12.

18 ALKALINITY OF SOLUBLE ASH—OFFICIAL

Proceed as directed under XXXIV, 13.

19 ALKALINITY OF INSOLUBLE ASH—OFFICIAL

Proceed as directed under XXXIV, 14.

20 ANALYSIS OF THE ASH—OFFICIAL

Proceed as directed under XII and XXVI.

21 PRESERVATIVES—OFFICIAL

Proceed as directed under XXXII.

22 COLORING MATTERS—TENTATIVE

Proceed as directed under XXI, 2 and 8–25 inc.

23 METALS—TENTATIVE

Proceed as directed under XXIX.

ANTHRANILIC ACID ESTER

*Colorimetric Method<sup>3</sup>—Official*

(Use when the sample contains less than 500 mg per liter.)

24 REAGENTS

(a) *Hydrochloric acid soln.*—Dilute 83 cc of HCl to 1 liter with H<sub>2</sub>O.

(b) *Sodium nitrite soln.*—Dissolve 2 g of NaNO<sub>2</sub> in 100 cc of H<sub>2</sub>O.

(c) *Hydrazine sulfate soln.*—Dissolve approximately 3 g of N<sub>2</sub>H<sub>4</sub>·H<sub>2</sub>SO<sub>4</sub> in 100 cc of H<sub>2</sub>O.

(d) *Sodium-α-naphthol-2-sulfonate soln.*—Dissolve 5 g of the sulfonate in 100 cc of H<sub>2</sub>O.

(e) *Sodium carbonate soln.*—Dissolve 25 g of Na<sub>2</sub>CO<sub>3</sub> in 75 cc of H<sub>2</sub>O.

(f) *Standard soln of methyl anthranilate.*—Dissolve 0.25 g of methyl anthranilate in 60 cc of 95% (by volume) alcohol and dilute with H<sub>2</sub>O to 250 cc.

## 25

## APPARATUS

(a) *Steam generator filled with H<sub>2</sub>O*.—An oil can holding 1 gallon will serve the purpose.

(b) *Distillation flask*.—Kjeldahl flask of about 750 cc capacity, with shortened neck, about 10 inches in height over all.

(c) *Spray tube*.—Glass tube with a small perforated bulb at the end. Passes thru a rubber stopper and reaches to the bottom of the distillation flask.

(d) *Connecting bulb*.—Kjeldahl bulb with bent connecting tube.

(e) *Worm condenser*.—Having a water jacket 10–12 inches long. The outlet tube is extended to reach into the bottom of the receiving flask.

(f) *Receiving flask*.—500 cc Erlenmeyer flask.

## 26

## DETERMINATION

Place enough H<sub>2</sub>O in the receiving flask to just cover or seal the end of the extended condenser tube. Place 10–100 cc of the sample of flavor in the distillation flask; add, if necessary, sufficient H<sub>2</sub>O to make the volume 100 cc; insert the stopper carrying the spray tube and connecting bulb; and connect with the condenser and receiving flask. Immerse the distillation flask in a water bath to the level of the contents, and when the sample has attained the temp. of the nearly boiling bath connect with the steam generator and pass a rapid current of steam thru the sample until about 300 cc of distillate has been collected.

Disconnect the apparatus and wash out the condenser with a little H<sub>2</sub>O. Add to the distillate 25 cc of the HCl soln and 2 cc of the NaNO<sub>2</sub> soln, mix well, and let stand for exactly 2 min. Add 6 cc of the N<sub>2</sub>H<sub>4</sub>·H<sub>2</sub>SO<sub>4</sub> soln and mix well for a min., so that the liquid comes in contact with all parts of the flask that may have been touched by the soln when it contained free nitrous acid. Keep the liquid in the flask in rapid motion, add quickly 5 cc of the Na- $\alpha$ -naphthol-2-sulfonate soln, and then add immediately 15 cc of the Na<sub>2</sub>CO<sub>3</sub> soln. Dilute the colored soln to 500 cc with H<sub>2</sub>O, mix, and compare the color of an aliquot with the color of a standard or set of standards, prepared as nearly as possible at the same time. Calculate and express results as mg of anthranilic acid ester, as methyl anthranilate, per liter of sample.

*Gravimetric Method*—Official

(Use when the sample contains 500 mg or more per liter.)

## 27

## REAGENTS

(a) *Hydrochloric acid soln*.—Dilute 83 cc of HCl to 1 liter with H<sub>2</sub>O.

(b) *Sodium nitrite soln*.—Dissolve 2 g of NaNO<sub>2</sub> in 100 cc of H<sub>2</sub>O.

(c)  *$\alpha$ -naphthol soln*.—Dissolve 0.2 g of  $\alpha$ -naphthol in 100 cc of 30% (by volume) alcohol.

(d) *Sodium bicarbonate soln*.—Dissolve 8.4 g of NaHCO<sub>3</sub> in 100 cc of H<sub>2</sub>O.

## 28

## APPARATUS

Use the apparatus described under 25.

## 29

## DETERMINATION

Place in the distillation flask a quantity of the sample of flavor that contains from 50–125 mg of anthranilic acid ester and dilute, if necessary, to 100 cc with H<sub>2</sub>O. Subject the sample to steam distillation as directed in 23, collecting about 400 cc

of distillate. Have the  $H_2O$  in the bath near the boiling point when the bath is placed under the distillation flask, also have the  $H_2O$  in the steam generator boiling, and make the connection immediately.

Wash out the condenser with a little  $H_2O$  and dilute the distillate to 500 cc. Mix, and to a 200 cc aliquot add 5 cc of the  $HCl$  soln, and 5 cc of the  $NaNO_2$  soln. Mix well and let stand for 1 min. Mix 25 cc of the  $\alpha$ -naphthol soln and 6 cc of the  $NaHCO_3$  soln, pour the diazotized soln into the mixture, and let stand for 10 min. Fold two Whatman No. 1 or S. & S. No. 595 filter papers, 12.5 cm in diameter, and determine the difference in their weights by placing one on each pan of the balance and counterpoising with added weights. Place the heavier inside the lighter paper, fit into a funnel, and moisten. Pour the mixture thru this filter and wash the precipitate 7 or 8 times, using a total of about 100 cc of  $H_2O$  for this purpose. Fill the filter only to within 1 cm of the top. Place the funnel carrying the filter and washed precipitate in an oven, and dry for about 10 min. at a temp. of  $100^\circ$ . Then separate the filter papers and dry them for about 1 hour at the same temp. Ascertain the difference in weights, dry again, weigh again, and repeat this procedure until the difference in weights remains constant. From this constant difference in weights subtract the original difference in weights of the 2 filter papers and multiply the result by 0.4935 to obtain the weight of anthranilic acid ester, as methyl anthranilate. Calculate and express as grams per liter of sample.

30

BENZALDEHYDE<sup>a</sup>—TENTATIVE

Measure into a distilling flask 500 cc of the beverage, 100 cc of flavoring sirup, or 10–25 cc of flavor; add 22 cc of alcohol, and in the case of the flavor, about 200 cc of  $H_2O$ , and proceed as directed in XVI, 55.

31

GAMMA UNDECALACTONE<sup>b</sup>—TENTATIVE

Proceed as directed under XVI, 47, using 500 cc of beverage, 100 cc of flavoring sirup, or 10–50 cc of flavor.

## SELECTED REFERENCES

<sup>1</sup> J. Assoc. Official Agr. Chem., **19**, 74 (1936).

<sup>2</sup> Ibid., 75.

<sup>3</sup> Ibid., **11**, 46 (1928).

<sup>4</sup> Ibid., 47.

<sup>5</sup> Ibid., **19**, 75 (1936).

<sup>6</sup> Ibid., **16**, 420 (1933); **19**, 75 (1936).



## XIV. MALT BEVERAGES, SIRUPS AND EXTRACTS, AND BREWING MATERIALS

### I. BEER

(Unless otherwise directed express results as g per 100 g.)

1

#### PREPARATION OF SAMPLE—OFFICIAL

Remove CO<sub>2</sub> by transferring the sample to a large flask and shaking gently at first and then vigorously, or by pouring back and forth between beakers, preferably brass, nickel, or copper (tin lined), and do not allow the temp. of the beer to fall below 20°. Eliminate the foam by passing the CO<sub>2</sub>-free beer thru a dry filter paper.

2

#### COLOR—TENTATIVE

Determine the depth of color of the sample in a  $\frac{1}{2}$  inch cell with a Lovibond tintometer, using Series 52 slide. Express the result in terms of a  $\frac{1}{2}$  inch cell. Use the standard daylight lamp, 45.

3

#### SPECIFIC GRAVITY—OFFICIAL

Determine the sp. gr. at 20/20° (in air) by means of a pycnometer as follows: Carefully clean the pycnometer by filling with a saturated soln of CrO<sub>3</sub> in H<sub>2</sub>SO<sub>4</sub>, allowing to stand for several hours, emptying, and rinsing thoroly with H<sub>2</sub>O. Fill the pycnometer with recently boiled H<sub>2</sub>O previously cooled to 2-4° below the desired temp., place in a water bath cooled to the same temp., and allow the bath to warm slowly to the desired temp. Adjust the level of the H<sub>2</sub>O to the proper point on the pycnometer; put the perforated cap or stopper in place, remove the pycnometer from the bath, wipe dry with a clean cloth, and after allowing to stand for 15-20 min., weigh. Empty, rinse several times with alcohol, and then with ether, remove the ether fumes, allow the pycnometer to become perfectly dry, and weigh. Ascertain the weight of contained H<sub>2</sub>O at the desired temp. in air (*W* of the formula below) by subtracting the weight of the empty pycnometer from its weight when full. Cool the sample to 2-4° below the desired temp., fill the pycnometer, adjust the level of the liquid to the proper point on the pycnometer at the desired temp., put the perforated cap or stopper in place, wipe dry, and weigh as before. Ascertain the weight of the contained sample at the desired temp. in air (*S* of the formula below) by subtracting the weight of the empty pycnometer from its weight when filled with the sample. Sp. gr. = Weight of contained beer ÷ Weight of contained water. Use the Plato table, 3, XLII, to ascertain apparent extract or saccharometer indication. Calculate the sp. gr. in vacuo, if desired, by the following formula:

$$G = \frac{S + 0.00105W}{W + 0.00105W'} \text{ in which}$$

*G* = corrected sp. gr. of sample at the desired temp. in vacuo;

*W* = weight of contained H<sub>2</sub>O at the desired temp. in air; and

*S* = weight of contained sample at the desired temp. in air.

4

#### APPARENT EXTRACT OR SACCHAROMETRIC INDICATION—TENTATIVE

From the Plato table, 3, XLII, ascertain the apparent extract corresponding to the sp. gr. determined at 20/20°.

## 5 ALCOHOL—OFFICIAL

(a) *By volume*.—Measure 100 cc of the liquid into a 300-500 cc distillation flask, noting the temp., and add 50 cc of H<sub>2</sub>O. Attach the flask to a vertical condenser by means of a bent tube, distil almost 100 cc, and make to a volume of 100 cc at the same temp. Determine the sp. gr. of the distillate as directed under 3, at room temp. if desired, and obtain the corresponding percentage of alcohol by volume from Table 19, XLII.

(b) *By weight*.—From Table 21, XLII, obtain the % alcohol by weight in the distillate corresponding to the % alcohol by volume and divide by the sp. gr.

(c) *By immersion refractometer*.—Verify the percentages of alcohol, as determined under (a) and (b), by ascertaining the immersion refractometer reading of the distillate and obtaining the corresponding percentages of alcohol from Table 20, XLII.

## 6 REAL EXTRACT—OFFICIAL

Calculate the sp. gr. of the dealcoholized beer by the following formula:

$S = G + 1 - A$ , in which

$S$  = the sp. gr. of the dealcoholized beer;

$G$  = the sp. gr. of the beer; and

$A$  = the sp. gr. of the distillate obtained in the determination of alcohol, 5.

From Table 3, XLII, ascertain the percentage by weight of extract in the dealcoholized beer corresponding to the value of  $S$ . The figure thus obtained  $\times S$  = the grams of extract per 100 cc of beer.

## 7 REAL EXTRACT—TENTATIVE

(a) Evaporate on the water bath or asbestos plate 75-100 cc of sample (accurately weighed to within 0.1 g) to about  $\frac{1}{3}$  of its original volume, but do not allow the temp. to exceed 80°. Cool, make up to original weight with H<sub>2</sub>O and determine sp. gr. with a pycnometer at 20°/20°. (If too much H<sub>2</sub>O has been added, the sp. gr. will be proportionately too low, and a correction must be made.) Ascertain the real extract directly from the Plato table, 3, XLII.

(b) Immersion refractometer reading of the beer at 20° minus the refractometer reading of the distillate at 20°  $\times 0.2571$  = the grams of extract in 100 cc of beer.

(c) If no anti-foam material was used in the determination of alcohol, 5, transfer the residue quantitatively with hot H<sub>2</sub>O to a 100 cc flask. Cool, and make up to 100 cc at 20°. Determine the sp. gr. at 20°/20°, 3, and ascertain the extract direct from the Plato table, 3, XLII. If 100 cc of beer was taken, make the following correction:

$$\text{Extract found} \times \frac{\text{sp. gr. of dealcoholized beer}}{\text{sp. gr. of beer}} = \text{g of extract in 100 g of beer.}$$

## 8 EXTRACT OF ORIGINAL WORT—TENTATIVE

Calculate from the following formula:

$$O = \frac{A \times 2.0665 + E \times 100}{100 + (A \times 1.0665)}, \text{ in which}$$

$O$  = Extract of original wort

$A$  = % alcohol by weight (g per 100 g of beer); and

$E$  = real extract, 6.

**9 REAL DEGREE OF FERMENTATION OR REAL ATTENUATION—TENTATIVE**

Calculate as follows:

$$\frac{\text{Orig. ext.} - \text{real ext.}}{\text{Orig. ext.}} \times 100.$$

**10 TOTAL ACID—TENTATIVE**

To 25 cc of beer heated to 40° to remove CO<sub>2</sub>, add about 1 cc of phenolphthalein indicator soln. Titrate immediately with 0.1 N alkali. With dark beers, dilute with H<sub>2</sub>O. Express the results as lactic acid, g per 100 cc; 1 cc of 0.1 N alkali = 0.0090 g of lactic acid.

**11 VOLATILE ACIDS—OFFICIAL**

Using 100 cc of beer, proceed as directed under XV, 24. Express the result as acetic acid, g per 100 cc.

**12 REDUCING SUGARS—OFFICIAL**

Dilute 25 cc of the prepared sample, 1, measured at 20°, to 100 cc with H<sub>2</sub>O at the same temp. Determine the reducing sugars in 25 cc of this soln as directed under XXXIV, 31 or 36. Express the result as grams of maltose per 100 cc of beer.

**13 DEXTRIN—TENTATIVE**

To 50 cc of the prepared sample, 1, measured at 20°, in an Erlenmeyer flask, add 15 cc of HCl (sp. gr. 1.125) and dilute to 200 cc. Attach the flask to a reflux condenser, and keep in a boiling water bath for 2 hours. Cool, nearly neutralize with NaOH, make up to a volume of 250 cc, filter, and determine dextrose as directed under XXXIV, 46 or 48. From the number of g of dextrose per 100 cc of beer, subtract 1.053 times the quantity of maltose, 12, and multiply the remainder by 0.9. The result is the number of g of dextrine per 100 cc of beer.

**14 DIRECT POLARIZATION—TENTATIVE**

Read the polarization of the original sample in °Ventzke in a 200 mm tube at 20°. If the beer is turbid, clarify by shaking with alumina cream, filter, and correct the reading for dilution.

**15 GLYCEROL—OFFICIAL**

Proceed as directed under XV, 6.

**16 ASH—OFFICIAL**

Evaporate to dryness 50 cc of the prepared sample, 1, measured at 20°, and proceed as directed under XXVII, 8.

**17 PHOSPHORIC ACID—OFFICIAL**

To 50 cc of the prepared sample, 1, measured at 20°, add 20 cc of 2% Ca acetate soln, evaporate to dryness, and ignite at low redness to a white ash. Add 10–15 cc of boiling HNO<sub>3</sub> (1+9) and determine P<sub>2</sub>O<sub>5</sub> as directed under II, 12.

**18 PROTEIN—OFFICIAL**

To 25 cc of the prepared sample, 1, at 20° in a Kjeldahl digestion flask, add 2–3 cc of H<sub>2</sub>SO<sub>4</sub> (1+1) and evaporate to dryness. Determine N as directed under II, 21, 23 or 25. Multiply the result by 6.25 to calculate the percentage of protein.

CARBON DIOXIDE<sup>2</sup>—TENTATIVE

19

## REAGENTS

*Sulfuric acid*.—Approximately 2.25 N. Standardize with 4–6 g of anhydrous  $\text{Na}_2\text{CO}_3$  dissolved in 200 cc of  $\text{H}_2\text{O}$ . Titrate to phenolphthalein and methyl orange end points. Use the difference between these two end points to calculate the titer of the acid (about 0.1 g of  $\text{CO}_2$  per cc).

(b) *Pyrex distilling head*.

Have all apparatus set up ready for immediate use.

20

## DETERMINATION

Remove the label and weigh the sample bottle. Cool the bottle and contents to  $0^\circ$  in a refrigerator or in chopped ice and allow to stand at rest overnight at this temp.

Avoid shaking when removing the bottle from the refrigerator or chopped ice. Remove the crown and immediately add 0.1–0.2 g of infusorial earth and 1–2 cc of hexyl alcohol or capryl alcohol. Quickly insert a rubber stopper provided with a distilling head, the outlet end of which is provided with a rubber connection closed with a screw clamp. Clamp the bottle in position in an empty liter beaker and connect with a 250 cc tall Drexel gas washing bottle, which contains 25 cc of 5 N KOH soln and 150 cc of  $\text{H}_2\text{O}$ . The orifice of the intake tube is restricted to 1 mm diameter at the point where the gas enters the KOH. Open and adjust the screw clamp to permit the gas to pass into the gas washing bottle at the rate of 3–4 bubbles per second. When the screw clamp is completely open and the gas evolution slows up, fill the beaker with  $\text{H}_2\text{O}$  and apply the full flame of the burner. Keep the  $\text{H}_2\text{O}$  in the beaker boiling vigorously and add boiling  $\text{H}_2\text{O}$  to replace loss by evaporation. Continue this process until the bubbles cease to come over and the alkaline liquid in the washing bottle rises in the inner tube to the level of the outer liquid.

Disconnect the washing bottle, cool, and transfer the contents to a 500 cc Erlenmeyer flask. Titrate slowly, imparting a rapid swirling motion to the contents of the flask, with the standard  $\text{H}_2\text{SO}_4$  soln, using 3 drops of indicator soln (0.6 g of thymolphthalein and 1 g of phenolphthalein in 60 cc of alcohol diluted with 40 cc of  $\text{H}_2\text{O}$  until the lavender color of the soln changes to phenolphthalein pink. Continue to a faint pink and note the buret reading.

Then add 2 drops of methyl orange indicator (0.2 g of methyl orange in 100 cc of  $\text{H}_2\text{O}$ ) and continue the titration to the methyl orange end point without refilling the buret. Correct the number of cc of standard acid required to pass from the phenolphthalein to the methyl orange end point for the blank due to carbonates inherent in the KOH soln, determined by titrating, in the same manner, 25 cc of the KOH soln in 150 cc of  $\text{H}_2\text{O}$ . During the titration between the two indicator changes the  $\text{CO}_2$  of the beer is present in the KOH soln as  $\text{KHCO}_3$ .

Disconnect the beer bottle from the apparatus, wash, drain, and allow to dry. Weigh the empty bottle and crown.

Calculate the weight of  $\text{CO}_2$  obtained by subtracting the blank from the difference in cc between the thymolphthalein and methyl orange end points and multiplying this difference by the titer of the acid.

Calculate the percentage weight of  $\text{CO}_2$  present as follows:

$$\% \text{ CO}_2 = \frac{\text{Weight of CO}_2}{\text{charge}} \times 100.$$

**21 SULFUR DIOXIDE—TENTATIVE**

To 200 cc of beer (not necessarily decarbonated) in a 1000 cc distilling flask, add 250 cc of  $H_2O$  and 1 cc of  $H_3PO_4$ . Add a pinch of  $Na_2CO_3$  and distil with steam. Collect about 200 cc of the  $SO_2$ -containing distillate in a flask containing 50 cc of saturated Br water or I soln; acidify with HCl, boil to remove Br or I and precipitate with  $BaCl_2$ . Weigh as  $BaSO_4$  and report as mg of  $SO_2$  per liter.

**22 IODINE REACTION<sup>1</sup> OR UNCONVERTED STARCH—TENTATIVE**

(a) 1.—Place 10 cc of beer in one test tube; 2.—In a second test tube place 0.1 N I soln (dissolve 12.69 g of I and 25 g of KI in  $H_2O$  and make up to 1 liter) and dilute to the same color as the beer. Slowly pour the I soln into the beer and note the color. A normal beer should not change in color. A blue color indicates starch; a purple color, amyloextrine; and a reddish color, erythrodextrine. No change in color indicates complete conversion.

(b) *For dark beer, but applicable also to light beer.*—To 5 cc of beer in a test tube add 25 cc of alcohol. Shake thoroly. Let stand. Decant, pouring off the last trace of the beer-alcohol mixture. Dissolve the precipitate (dextrine) in 5 cc of  $H_2O$  and to this soln add dropwise a 0.1 N I soln diluted 5 times. Abnormal beer gives a red or violet or blue coloration.

**23 COLORING MATTERS—TENTATIVE**

Proceed as directed under XXI.

**24 METALS—TENTATIVE**

Proceed as directed under XXIX.

**25 PRESERVATIVES AND SWEETENERS—OFFICIAL**

Proceed as directed under XXXII.

**26 PASTEURIZATION—TENTATIVE**

Beer heated to  $58^\circ$  (temp. of pasteurization) no longer contains active invertase. Take two 20 cc portions of beer, one heated to boiling, the other not heated. To each add 20 cc of a 20% sucrose soln and let stand 24 hours at room temp. Add to each soln 0.5 cc of basic Pb acetate soln, make up to 50 cc with  $H_2O$ , filter, and polarize. If there is an appreciable difference in rotatory power between the two samples the beer has not been pasteurized. A close agreement in rotation indicates a pasteurized beer.

**27 CHLORIDES IN BEER<sup>1</sup>—TENTATIVE**

Take a 100 cc sample and proceed as directed under XII, 34–36.

**28 METHYL ALCOHOL (QUANTITATIVE)—TENTATIVE**

Transfer to a distilling flask a quantity of sample that contains 20–25 cc of absolute alcohol and distil slowly, collecting the distillate in a 50 cc volumetric flask. When nearly to the mark, disconnect the receiver and adjust to the mark at room temp. with  $H_2O$ . Determine methyl alcohol as directed under XVI, 19.

**MALT\*—TENTATIVE****29 SAMPLING**

For complete descriptions of the trier, divider, sampler, and bushel weight tester, see Handbook of Official Grain Standards of the United States Department of Agriculture, 1934.

\* These methods are the official methods for analysis of malt adopted by the American Society of Brewing Chemists, October, 1935, edited to conform in part to the style of this publication.

(a) *Bulk malt in cars or bins.*—Using a 60-in. trier, take at least 6 probes from different parts of the car, preferably 2 from the center and 2 from each end.

(b) *Bulk malt during discharge through spouts or openings.*—At different times during the filling or unloading of a car take, with the trier or sampler, at least 6 samples, each representing a complete cross section from the grain stream coming out of the spout.

(c) *Bagged malt.*—Sample not less than 2% of the bags, lengthwise thru the center of the open bags, using a short trier.

Indicate the approximate proportion of inferior grain and take representative samples from each portion in the manner outlined above. Immediately place each portion of the sample in a suitable large dry container and keep tightly closed.

## 30

## PREPARATION OF SAMPLE

Divide the samples either by quartering or by using a sample divider, until approximately 1 lb. remains. Place the reduced sample in an air-tight container (preferably tin with a screw or friction type cover). Do not use cartons, bags, wooden boxes, glass Mason jars, or wrapping paper for this purpose. Remove foreign particles, such as stone, wood, and twine. Do not remove foreign seeds or dust particles.

## 31

## BUSHEL WEIGHT

Place the sample in the filling hopper of the Winchester tester, open the slide underneath, and allow the malt to fill the measuring cylinder to overflowing. Without jarring, level off with a straight-edge longer than the diameter of the measuring cylinder, making one forward stroke consisting of three distinct zig-zag motions. Weigh to the nearest  $\frac{1}{4}$  pound.

## 32

## LENGTH OF ACROSPIRE

Quarter the sample until approximately 200 kernels remain in two opposite quarters. Count out 100 kernels, remove the husk covering the acrospire by means of a sharp instrument (knife or tweezer with sharpened edge), and note the acrospire length in comparison with the length of the kernel. Divide the kernels into the following classifications: 0 to  $\frac{1}{4}$ ,  $\frac{1}{4}$  to  $\frac{1}{2}$ ,  $\frac{1}{2}$  to  $\frac{3}{4}$ ,  $\frac{3}{4}$  to 1, overgrown. Include  $\frac{1}{4}$ ,  $\frac{1}{2}$  and  $\frac{3}{4}$  grown kernels in the upper classification. Count the number of kernels belonging to each class.

## 33

## MEALINESS

Count out 100 kernels remaining from the preceding test and cut them in longitudinal halves. Determine the percentage of mealy, half glassy, and glassy kernels. In case of uncertainty, pierce the starch body with a sharp point; if mealy, it will break away and crumble from the point.

## 34

## 1,000 KERNEL WEIGHT

Quarter the sample until approximately 500 kernels remain in two opposite quarters. Count out 500 kernels and weigh to the nearest 0.1 g. Calculate the results to 1,000 kernels on "as is" and dry basis.

## 35

## ASSORTMENT\*

Weigh 100 g from a quartered sample to the nearest 0.1 g. Place in the top compartment of a grader and shake for 3 min. Weigh the quantities remaining on the

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\* Grader manufactured by the Richmond Mfg. Co., Lockport, N. Y.

various screens and in the catch pan to the nearest 0.1 g, and report the percentage on each of the following screens: 8/64", 7/64", 6½/64", 5½/64", 5/64", and thru the 5/64", in percentage totaling 100%.

36

## MOLD

Determine the presence or absence of mold by visual inspection and report as "none," "trace," etc.

37

## FOREIGN SEEDS AND BROKEN KERNELS

Weigh 50 g of the sample. Pick out foreign seeds and broken kernels, classify, and report separately in percentage.

## MOISTURE

38

## APPARATUS

(a) *Weighing dish*.—Use a glass bottle or an aluminum dish, provided with a tight fitting cover, and having a diameter of approximately 40 mm for a 5 g sample, or 55 mm for a 10 g sample.

(b) *Oven*.—Should have an automatic temp. control capable of holding the temp. within  $\pm 0.5^{\circ}$  C. and be large enough to accommodate all the samples on one shelf in such a manner that no sample is outside the area indicated by test to give comparable results in duplicate samples. Standardize the oven as follows: Place weighed duplicate samples in the oven at 103 to 104° and dry for 3 hours. Weigh, and redry for 1 hour longer. If the moisture content has increased by more than 0.1%, raise the temp. 1° and again test with new duplicate samples. Take as the standard the lowest temp. below 106° giving a moisture content which, after 3 hours of drying, is within 0.1% of the value attainable at the same temp. within 4 hours. Keep the ventilators of the oven open during the entire drying period, and do not open the door during the 3 hours of drying.

39

## PREPARATION OF SAMPLE

(a) *If extract determination is to be made*.—Grind as instructed under "Extract". and transfer in one continuous operation. When a large number of samples are used, grind the first sample, remove the beaker, and grind the second sample while adjusting the weight of the first sample. Remove the second sample, insert the third sample, and repeat the operation.

(b) *If extract determination is not made*.—Have the sample of the same fineness as the finely ground malt used for the determination of extract. Weigh about 5 g of whole malt and grind thru a clean dry mill directly into a weighing bottle (10 g may be taken provided the 55 mm diameter weighing bottle is used.) Brush all malt from the mill into the weighing bottle and cover immediately.

40

## DETERMINATION

Weigh the sample to 0.1 mg and place in the oven previously heated to the standard temp. Remove the cover of the weighing bottle and heat for exactly 3 hours at the standard temp. Transfer the weighing bottle, with the cover replaced, to a desiccator and when cooled to room temp. weigh to 0.1 mg. Report moisture to the nearest 0.1%.

## EXTRACT

41

## REAGENT

*Iodine soln.*—0.01 N. Dissolve 0.63 g of I and 1.25 g of KI in H<sub>2</sub>O and make up to 500 cc. Prepare a fresh soln every month.

(a) *Mill*.—Miag-Seck. For fine grinding use the cone-type, 300 r.p.m., and for coarse grinding the roller-type, 150 r.p.m.

(b) *Sieves*.—Set of six 8", half-height U. S. standard sieves Nos. 10, 14, 18, 30, 60, and 100 (with pan and cover).

(c) *Mash beakers and counter weights*.—Made of either pure nickel or brass, not copper, and of such dimensions as to assure a tight connection between the beakers and the Miag-Seck mill during the grinding period.

If counter weights are used for the mash beakers, check their tare weight frequently.

(d) *Mashing apparatus*.—Beakers, stirrers, and solder shall be made of the same metal. Each stirrer shall be provided with a blade, which in operation has a clearance of about 2 mm from the bottom and 5 mm from the wall of the mash beaker. The blade shall be about 8 mm wide, and each side shall have a pitch of 45°, arranged as in a propeller, to cause an upward motion of the mash. The speed of the mash stirrer shall be 80-100 r.p.m., each stirrer of each beaker having the same speed. Stir the H<sub>2</sub>O in the bath thoroly by mechanical means to assure uniformity of temp. and have the level of the H<sub>2</sub>O above the maximum mash level.

(e) *Gypsum plate*.—Prepare the gypsum plate by thoroly mixing 100 cc of H<sub>2</sub>O with 135 g of plaster of Paris. Pour this mixture, while still free-flowing, into suitable flat molds (cigar boxes, etc.).

(f) *Filter paper*.—Use Schleicher and Schüll 32 cm fluted filter paper No. 560 (or No. 597, 32 cm to be fluted by the analyst) or Delta Düren, 32 cm fluted filter paper No. 314½ (or No. 314, 32 cm, to be fluted by the analyst).

(g) *Funnels*.—Use short-stem glass funnels of approximately 20 cm diameter and do not allow the filter paper to project above the rim of the funnel. The stem shall extend about 3-5 cm into the receiving flask.

(h) *Flasks*.—Use dry 500 cc Erlenmeyer flasks. Mark at the 100 cc level.

(i) *Pycnometers*.—Any suitable pycnometer but preferably of the Reischauer type, which is approximately 15 cm high and has a neck approximately 9 cm long and an internal neck diameter between 2.5 and 3.5 mm. Place a thin, well defined mark 55-70 mm below the upper rim of the neck. When filled with H<sub>2</sub>O of 20° it shall have a capacity of 48-50 g. Use glass funnels having a capacity of about 15 cc to fill the pycnometers.

(j) *Emptying device for Reischauer pycnometer*.—Bend a piece of non-ferrous metal capillary tubing (brass, stainless steel) of less than 2 mm outside diameter to an angle of about 45°. The end to be inserted into the pycnometer shall reach within a few mm of the bottom. Connect the other end either to a rubber aspirator bulb or to a compressed air supply, not exceeding 5 lbs. per sq. in.

(k) *Water bath*.—Use an automatically controlled water bath. If one is not available, use the following set-up: Have the water level of the bath (5-15 liters capacity) reach above the neck marks of the pycnometer, keep the water bath temp. at 20° ( $\pm 0.05^\circ$ ), and read on an accurate thermometer, calibrated to 1/10°. Maintain the temp. of the water bath by a very slow but continuous flow of ice water from a container (2-4 liters capacity, containing ice and H<sub>2</sub>O). Regulate the flow of ice water by hand. Stir the H<sub>2</sub>O in the bath mechanically and continuously without splashing.

(a) *Setting of the mill*.—Determine by the composition of the ground malt and use malt of the following characteristics:



Moisture .....	4.0-5.0%
Extract in the finely ground malt (Plato) as is .....	68-71%
Color of laboratory wort, Lovibond series 52- $\frac{1}{2}$ " cell .....	1.5-2.2°
1,000 kernel weight, as is .....	22-26g

Acrospire development: 80% of the kernels to have an acrospire longer than  $\frac{1}{4}$  of kernel length; not more than 5% of the kernels to have acrospires longer than kernel length.

*Fine grinding.*—Weigh approximately 51 g. of the specified malt into a mash beaker, grind, and collect in the same beaker. Sieve 50 g of the well-mixed ground malt thru the standard sieves. Shake the sieves with the malt by hand in a horizontal plane for 5 min., tapping the set of sieves upon the table top every 15 seconds. Detach the top sieve from the other sieves and shake for a short time over a sheet of paper until no particles are emitted. Add the particles to the next sieve. Repeat this procedure with sieves Nos. 14, 18, and 30. The mill shall be considered as having a standardized setting when the sum of the ground malt portions remaining on sieves Nos. 10, 14, 18, and 30 is between 4.5 and 5.5 g (9-11%). Standardize the mill at least twice yearly.

*Coarse grinding.*—Proceed as directed for *fine grinding*. The mill shall be considered as having a standardized setting when the sum of the ground malt portions remaining on sieves Nos. 10, 14, 18, and 30 is between 29 and 31 g (58-62%).

(b) *Pycnometers.*—Clean the interior and exterior of the pycnometers with  $\text{Na}_2\text{Cr}_2\text{O}_7\text{-H}_2\text{SO}_4$  soln, discharge carefully with air, and wash several times with  $\text{H}_2\text{O}$ , followed with alcohol and finally with ether. To remove the last traces of ether vapor and replace it with laboratory air, connect the metal capillary tubing (dry) to a vacuum and insert into the pycnometer for 1-2 min. Carefully wipe the pycnometers, allow to stand a few minutes, and determine the tare by weighing to 0.2 mg.

Fill with freshly distilled  $\text{H}_2\text{O}$  and place in the water bath held at  $20^\circ (\pm 0.05^\circ)$ . Force out air bubbles by gentle tapping. After 25 min., remove the liquid above the mark by means of a capillary pipet provided with a small rubber bulb. Make the final adjustment of the meniscus by absorbing the last quantity of liquid by means of thin strips of blotting paper; also remove any liquid adhering to the inner surface of the neck. Adjust the water level so that the lower part of the meniscus rests on the mark. Make all adjustments of the liquid level within the pycnometer neck while holding it at the neck, but without touching the body of the pycnometer with the hands. Keep the body of the pycnometer submerged during the entire period of the meniscus adjustment.

Raise the pycnometers to room temp. by insertion into a water bath, kept at exactly that temp., and hold for 10 min. Remove the pycnometers, carefully dry the exterior, and weigh to 0.2 mg. The difference between the two weighings represents the  $\text{H}_2\text{O}$  capacity of the pycnometer at  $20^\circ$ . Redetermine the tare weight and the  $\text{H}_2\text{O}$  capacity at least twice a year.

## 44

## DETERMINATION

*Fine grinding.*—Weigh approximately 55 g of the sample (at room temp.) into a tared mash beaker and grind thru the mill set for standardized fineness of grind. Collect the finely ground malt in the same mash beaker, carefully brushing malt particles remaining in the mill into the mash beaker. Without delay, place the mash beaker with its contents on the balance (accurate to within  $\pm 0.05$  g under a 750 g load) and adjust weight of malt to 50 g ( $\pm 0.05$  g) by removing the excess into a tared dish for the determination of moisture.

*Coarse grinding.*—Weigh 50.5 g or less of the sample (at room temp.) into a tared mash beaker and grind thru the mill, set for standardized coarseness of grind. Collect the coarsely ground malt in the same mash beaker, carefully brushing particles remaining in the mill into the mash beaker. Without delay, place the mash beaker with its contents on the balance (accurate to within  $\pm 0.05$  g under a 750 g load) and adjust weight of malt to 50 g ( $\pm 0.05$  g) by removing the excess.

(a) *Mashing procedure.*—"Mash in" the ground malt with 200 cc of  $H_2O$  of  $46^\circ$  and mix well with a glass rod to prevent formation of lumps. Carefully rinse the glass rod and the wall of the beaker with a small quantity of  $H_2O$ . Note the odor of the mash and report as aromatic, slightly aromatic, musty, green, stale, etc. Promptly place the mash beakers in the mashing apparatus containing  $H_2O$  previously heated to  $46^\circ$ , and set the stirrers in motion. Place a thermometer in each mash beaker. Maintain a temp. of  $45^\circ$  for exactly 30 min. from the time the beakers were placed in the mashing apparatus. Raise the mash temp.  $1^\circ$  per min. until  $70^\circ$  is reached. Add 100 cc of  $H_2O$ , previously heated to  $70$ – $71^\circ$ , and hold the mash at  $70^\circ$  for 60 min. (Temp. deviations during the mashing procedure should not exceed  $0.5^\circ$ .)

(b) *Conversion.*—Transfer a drop of mash by means of a thin glass rod (about 3 mm diameter) onto the absorbent gypsum plate, and test with a drop of the I soln, 41. Make tests 5, 7, and 10 min. after  $70^\circ$  has been reached, and thereafter, if necessary, at 5 min. intervals. Conversion is complete when the test drop and I produce only a yellow stain on the gypsum plate. Report the time of conversion in periods: less than 5 min., 5–7 min., etc. Time of conversion is not determined on coarsely ground malt.

(c) *Cooling and filtration.*—Cool the mash promptly within 10–15 min. to the prevailing room temp. Stop the stirrers. Remove the thermometers after mash particles adhering have been rinsed into the beaker with  $H_2O$ . Remove each beaker with its stirrer from the mashing apparatus. Rinse mash particles adhering to the stirrer into the beaker with  $H_2O$ . Dry the outside of each beaker, taking care to remove moisture adhering to the rim. Without delay, adjust the weight of the contents of the mash beaker to 450.0 g ( $\pm 0.05$  g) by the addition of  $H_2O$ .

Stir the mash thoroly with a glass rod, once when removing the beakers from the balance pan, and again immediately before pouring the mash onto the filter. The two stirrings shall be not less than 5 min., nor more than 15 min. apart. During the stirring of the cooled mash, take care to prevent splashing or spilling of the mash. Mix drops adhering to the beaker wall into the mash by rotary stirring with the glass rod.

Pour the entire contents of the beaker into a funnel provided with the specified filter paper. Cover the funnel with a large watch-glass (about 20 cm diameter) during the entire filtration. Return the first 100 cc of the filtrate to the filter. When no more liquid is present above the filter cake, discontinue filtration and remove the receiving flask, containing the wort, for subsequent observations and tests. In the case of slow running worts, stop filtration after 2 hours. When filtration is complete, mix the wort in the receiving flask thoroly by rotary motion. The speed of filtration is normal, if filtration is complete (as defined above) within 1 hour after returning the 100 cc filtrate to the filter bed; slow, if filtration requires longer. Observe the degree of clarity and report as: clear, slightly hazy, or hazy.

Remove approximately 100 cc of the wort for the determination of color. The color is not determined on the wort from coarsely ground malt.

(d) *Specific gravity.*—Rinse the empty pycnometer twice with about 10 cc of

wort, and if a Reischauer pycnometer is used remove the rinsings each time by means of the emptying device. Fill with wort, place in the water bath, and follow the procedure under 43(b). Weigh the filled pycnometer within 3 hours of completed filtration. The difference between this weight and that of the empty pycnometer represents the wort capacity of the pycnometer at 20°. Calculate the sp. gr. of the wort to the fifth decimal place, rounding off to 0.00005 or 0.00010, by dividing the weight of the wort by the weight of the H<sub>2</sub>O.

No calculation is made of sp. gr. in vacuo. If duplicate determinations made by the same analyst in different beakers differ by more than two units in the fourth decimal place, repeat the entire determination.

(e) *Extract*.—Ascertain the extract yield of the malt by reference to the sp. gr. values in XLII, Table 3, and report only to the first decimal place.

## 45

## COLOR OF WORT

Use a Lovibond tintometer,  $\frac{1}{2}$  in. cell, Series 52, brewers' type, and a standard daylight lamp (A.S.T.M., D 218-34T, 1933, or its spectrophotometric equivalent). Place the tintometer in a box shield of metal or wood, but finished in dull black so as to prevent interference from reflected light. Mount in a horizontal position directly in front of the artificial daylight lamp. Substitute a flashed, opal glass for the milk glass usually provided with the instrument. Have the distance between the opal glass and the daylight lamp such as to project a diffused light with the absence of glare or shadow upon the opal glass and have the near surface of the daylight filter 6 inches from the opal glass.

Pour the wort into the cell as quickly as possible after filtration and match against the standard glasses. Subdivide down to  $\frac{1}{2}^\circ$  color glasses and report results to the nearest tenth. If difficulty is experienced in reading the color, filter that portion of the wort to be used for color determination separately thru dry filter paper without filter-aid.

## DIASTATIC POWER

Wash all glassware with acid cleaning soln, 43(b), then rinse with ordinary tap H<sub>2</sub>O not less than 4 times, and finally rinse with distilled H<sub>2</sub>O at least twice. Thoroughly dry the digestion flasks.

## 46

## REAGENTS

(a) *Acetate buffer soln*.—Dissolve 68 g of Na acetate (CH<sub>3</sub>COONa·3H<sub>2</sub>O) in 500 cc of normal acetic acid and make up to 1 liter with H<sub>2</sub>O.

(b) *Soxhlet's modification of Fehling's soln*.—Prepare by mixing immediately before use equal volumes of (1) and (2).

(1) *Copper sulfate soln*.—Dissolve 34.639 g of CuSO<sub>4</sub>·5H<sub>2</sub>O in H<sub>2</sub>O, dilute to 500 cc, and filter thru prepared asbestos.

(2) *Alkaline tartrate soln*.—Dissolve 173 g of Rochelle salt and 50 g of NaOH in H<sub>2</sub>O, dilute to 500 cc, allow to stand for 2 days, and filter thru prepared asbestos.

Check the Fehling's soln from time to time by estimating its oxidizing value against a standard soln of invert sugar according to customary analytical procedure.

(c) *Starch soln*.—Have the final concentration represent 2 g of soluble starch (weighed on a dry basis) in 100 cc of soln. (Use starch of such quality and grade that its solubility will be at least 1:50 in hot H<sub>2</sub>O, contain no dextrines, contain less than 0.5% reducing substances calculated as maltose, and have a moisture content of approximately 10-12%. A freshly made 2% soln shall have a pH between 4.5 and 4.7 without adjustment by the use of a buffer. Subsequent batches of starch shall,

when tested on a malt of approximately 100° Lintner (dry basis) and having other characteristics as specified under the determination of extract in malt, show a variation no greater than  $\pm 3^\circ$  Lintner from the value obtained using the original starch in a parallel determination. Further additional batches of starch when purchased shall be tested in parallel with the starch in use. No variation greater than  $\pm 3^\circ$  Lintner will be permitted. In no case shall a cumulative correction as referred to the original starch approved above amount to more than  $5^\circ$  Lintner.) Macerate the starch with a small amount of cold freshly distilled  $H_2O$  sufficient to form a smooth thin paste (not over 5% of final volume). Pour this, with constant stirring, into boiling freshly distilled  $H_2O$  representing not less than approximately 75% of the final volume of the starch soln. at such a rate that boiling does not cease. Continue boiling for 2 min. after the thin paste is completely introduced. Quickly add an additional 10% of the final volume of cold freshly distilled  $H_2O$  to the beaker and transfer the mixture quantitatively to a glass-stoppered volumetric flask, mix by inverting the flask, wash down the neck of the flask, and cool the whole to  $20^\circ$  before adding the buffer soln. Add 2 cc of the buffer soln for each 100 cc of the final volume of starch soln and make up the whole to the mark. Mix again by inverting the flask and keep tightly stoppered at  $20^\circ$  until used.

## 47

## DETERMINATION

Grind separately not over 25.5 g of malt as directed under 44. Collect the finely ground malt in a mash beaker, carefully brushing in the malt particles remaining in the mill. Without delay, adjust the weight of the contents to 25 g ( $\pm 0.05$  g). Transfer quantitatively the 25 g to the container (capacity about 1 liter) in which the infusion is to be made. Add 500 cc of freshly distilled  $H_2O$  and close the container. Let the infusion stand for 2.5 hours at  $20^\circ$  ( $\pm 0.2^\circ$ ) and agitate by rotation at 20 min. intervals. Take care that in the agitation of the malt suspension as small a quantity as possible of the grist is left adhering to the inner surface of the flask above the level of the  $H_2O$ . (Mixing by inverting the flask is specifically cautioned against. Gentle whirling of the contents without splashing on the sides of the container has been found to give sufficient mixing.) Filter the infusion by transferring the entire charge onto a 30–32 cm fluted filter (CS and S No. 588) contained in a 175 mm funnel. Return the first 50 cc of the filtrate to the filter. Collect the filtrate until 3 hours shall have elapsed from the time the  $H_2O$  and ground malt were first mixed. Prevent evaporation during the filtration period as far as possible by placing a watch-glass over the funnel and some suitable cover around the stem of the funnel, resting on the neck of the receiver.

Immediately dilute 20 cc of the above infusion to 100 cc at  $20^\circ$ , transfer 10 cc of this infusion to a 200 cc volumetric flask, and bring to  $20^\circ$ . If the diastatic power of the malt being examined is  $135^\circ$  Lintner or above, make (or repeat) the determination, using a 250 cc volumetric flask at this point and 200 cc of the buffered starch soln. Multiply the diastatic power as computed under 48 by 1.25. Add 100 cc of buffered starch soln from a fast flowing pipet at  $20^\circ$ . Mix the solns by rotating the flask during the addition. Maintain the "starch-infusion" mixture at  $20^\circ$  ( $\pm 0.2^\circ$ ) for exactly 30 min. after addition of the starch soln was begun. Add 10 cc of 0.5 N NaOH rapidly for each 100 cc of starch soln and mix the whole thoroly by whirling the flask. Make to the mark at  $20^\circ$  and mix well.

Boil 10 cc of the Fehling's soln and 10 cc of  $H_2O$  in a small flask with a narrow neck (200 cc Erlenmeyer). Add from a buret about  $\frac{2}{3}$  of the amount of the above digested starch soln probably required and boil 15–20 seconds, rotating constantly.

Remove from flame. If still decidedly blue, add more soln, boil about 10 seconds, and again observe color. When the blue color has been almost discharged, and after boiling gently for about 2 min., add 3 drops of a 1% aqueous methylene blue soln. Continue boiling and add more soln until 0.1 cc, or even 1 drop, upon boiling, discharges the blue color. (It becomes violet-lavender as end point nears.)

Repeat the titration, adding at once almost the whole amount of the digested starch required in the above, and proceed to the end point as directed. Let the amount of the digested starch soln required to reach the end point in this second titration be called *A*. Interrupt the boiling as little as possible after the indicator has been added, so that the flask remains filled with steam, preventing much access of air. Upon cooling the blue color usually returns.

## 48

## BLANK CORRECTION

Prepare a blank by proceeding exactly as described under 47, except to add the NaOH to the malt infusion before adding the starch soln. Add to 10 cc of the Fehling's soln and 10 cc of H<sub>2</sub>O a volume of this blank equal to the final volume of digested starch soln required in the above determination. Boil and again determine the end point, using the *digested* starch soln, as directed under 47. Let the amount of digested starch soln used here be called *B*.

To determine the *corrected* diastatic power solve the formula  $\frac{4000}{A} \times \frac{B}{A} = \text{D. P.}$

in which  $\frac{4000}{A}$  is the apparent diastatic power, which must be modified by the

fraction representing the ratio of the blank titration to the original titration which measures the influence of the starch in the determination. To convert this to "dry basis," divide the figure so found by (100 minus per cent moisture). Report as degrees Lintner (dry basis).

### PREPARED CORN OR RICE PRODUCTS (FLAKED CORN OR FLAKED RICE)—TENTATIVE

## 49

## MOISTURE

Weigh out about 5 g in a glass weighing bottle or Al dish with tightly fitting cover, 40 mm in diameter, and dry at 103–104° for 4 hours. Record results to nearest first decimal.

## 50

## FAT

Extract the sample from the moisture determination with anhydrous ether for 5–6 hours, distil off the ether, and dry in an oven for 1 hour. Cool, and weigh.

## 51

## EXTRACT

To 30 g of finely ground barley-malt contained in a weighed mash beaker, add 200 cc of H<sub>2</sub>O at 46°. Mix malt and H<sub>2</sub>O with a glass rod, washing off rod and sides of beaker with a little distilled H<sub>2</sub>O. Maintain the temp. of 45° with constant stirring, preferably in a mashing machine, for 30 min. If no mashing apparatus is available, place beaker on a wire screen contained in a water bath. Raise the temp. 1° per min. until 67° is reached. Add 20 g of the prepared flaked corn or rice product, mix thoroly, and hold for 30 min. at 67°. Warm up to 70° in 6 min. Hold until

saccharified, testing every 3 min. by taking a small portion of the mash with a thin glass rod and placing this on a gypsum plate together with a drop of 0.01 *N* I soln, 41. A yellow coloration indicates complete inversion. Note the time after reaching 70° until mash is completely inverted. Hold altogether at 70° for 60 min. Cool to room temp., remove beaker from bath, wash off thermometer or stirrer with a little H<sub>2</sub>O, dry beaker, and adjust contents to 450 g. Stir contents of beaker thoroly and filter thru a fluted filter. Pour the first 100 cc of filtrate back on the filter, collecting the entire filtrate in a 500 cc Erlenmeyer flask. Determine the sp. gr. with a Reischauer or other pycnometer at 20° and find corresponding extract in the Plato Table, XLII, 3.

Calculate the extract as follows:

$$\text{Total extract} = \frac{\text{Extract} \times (800 + W \text{ in } 60 \text{ g of malt} + W \text{ in } 40 \text{ g of flakes})}{100 - \text{Extract}} \text{ in}$$

which

Extract = extract from Plato's table;

*W* = moisture;

$$\text{Extract in flakes} = \frac{\text{Total extract} - \text{extract in } 60 \text{ g of malt}}{40}.$$

## CORN GRITS, CORN MEAL, BREWER'S RICE<sup>1</sup> - TENTATIVE

52

### PREPARATION OF SAMPLE

If necessary, grind to fairly fine consistency.

53

### MOISTURE

Weigh out about 5 g in a glass weighing bottle or Al dish 40 mm in diameter with a tightly fitting cover, and dry at 103–104° for 4 hours. Record results to nearest first decimal.

54

### FAT

Extract the sample from the moisture determination with anhydrous ether for 5–6 hours, distil off ether, and dry in an oven for 1 hour. Cool, and weigh.

55

### EXTRACT

The extract and moisture content of the malt used must be known.

Boil for 30 min. 20 g of finely ground sample in a mash beaker with 200 cc of H<sub>2</sub>O, stirring with a glass rod and replacing the evaporated H<sub>2</sub>O. Cool to 46°, and add 30 g of crushed malt. Mix, and wash off glass rod and sides of beaker with a little H<sub>2</sub>O. Maintain the temp. of 45° for 30 min. with constant stirring, preferably in a mashing apparatus. If no mashing apparatus is available, place beaker on a wire screen contained in a water bath. Raise temp. 1° every minute until 70° is reached, and hold at this temp. until saccharified, testing every 3 min. by taking a small portion of the mash with a thin glass rod and placing this on a gypsum plate together with a drop of 0.01 *N* I soln, 41. A yellow coloration indicates complete inversion. Note the time after reaching 70° until mash is inverted. Hold altogether at 70° for 30 min. Cool to room temp., remove beaker from bath, wash off stirrer and thermometer with a little H<sub>2</sub>O, dry beaker, and adjust contents of beaker to 450 g. Stir with a glass rod and filter thru a fluted filter paper. Pour the first 100 cc of filtrate back on the filter collecting the entire filtrate in a 500 cc Erlenmeyer

flask. Determine the sp. gr. with a Reischauer or other pycnometer at 20° and find corresponding extract in the Plato Table, **XLII, 3**. Calculate the extract as directed under 51.

**56****REFINED GRITS AND REFINED FLAKES**

Proceed as directed for the examination of Corn Grits except to boil for 5 min. only in the determination of the extract.

**SELECTED REFERENCES**

<sup>1</sup> J. Assoc. Official Agr. Chem., 19, 76 (1936).

<sup>2</sup> Ibid., 77, 164.

## XV. WINES

1

### PHYSICAL EXAMINATION—TENTATIVE

Note and record the following: (1) Whether the container is "bottle full"; (2) the appearance of the wine, whether it is bright or turbid and whether there is any sediment; (3) condition when opened, whether still, gaseous, or carbonated; (4) color and depth of color; (5) odor, whether vinous, foreign or acetous, and (6) taste, whether dry, sweet, vinous, foreign, or acetous.

2

### PREPARATION OF SAMPLE—OFFICIAL

Remove any gas in the wine by pouring the sample back and forth in beakers. Filter the wine, regardless of appearance. Determine immediately the sp. gr. and those ingredients that are subject to change, such as alcohol, sugars, acids.

3

### SPECIFIC GRAVITY

Determine the sp. gr. at 20/20° by means of a pycnometer as directed under XIV, 3, or by means of a small accurately graduated hydrometer.

4

### ALCOHOL—OFFICIAL

(a) *By volume*.—Measure 100 cc of the liquid into a 300–500 cc distillation flask, noting the temp., and add 50 cc of H<sub>2</sub>O. Attach the flask to a vertical condenser by means of a bent tube, distil almost 100 cc, and make to a volume of 100 cc at the same temp. (Foaming, which sometimes occurs, especially with young wines, may be prevented by the addition of a small quantity of tannin.) To determine the alcohol in wines that contain an abnormal quantity of acetic acid, exactly neutralize the portion taken with normal NaOH soln calculated from acidity before proceeding with the distillation. (This is unnecessary, however, for wines of normal taste and odor.) Determine the sp. gr. of the distillate as directed under XIV, 3, at room temp. if desired, and obtain the corresponding percentage of alcohol by volume from XLII, Table 19.

(b) *By weight*.—From Table 21 obtain the % alcohol by weight in the distillate corresponding to the % alcohol by volume and divide by the sp. gr.

(c) *By immersion refractometer*.—Verify the percentages of alcohol (a) and (c), by ascertaining the immersion refractometer reading of the distillate and obtaining the corresponding percentages of alcohol from XLII, Table 20.

### GLYCEROL IN DRY WINES

At no time during any of the evaporations should the area of the dish exposed to the bath be greater in circumference than that covered by the liquid in the dish (easily done by allowing the dish to float in the bath).

5

### *I. By Direct Weighing—Official*

Evaporate 100 cc of the wine in a porcelain dish on a water bath maintained at a temp. of 85–90° to a volume of about 10 cc. Treat the residue with about 5 g of fine sand and 4–5 cc of milk of lime (containing 15 g of CaO per 100 cc) for each g of extract present and evaporate almost to dryness. Treat the moist residue with 50 cc of alcohol, 90% by volume; remove the substance adhering to the sides of the dish with a spatula, and rub the whole mass to a paste. Heat the mixture on a water bath, with constant stirring, to incipient boiling and decant the liquid thru a filter into a



small flask. Wash the residue repeatedly by decantation with 10 cc portions of hot 90% alcohol until the filtrate amounts to about 150 cc. Evaporate the filtrate to a sirupy consistency in a porcelain dish, transfer the residue to a small glass-stoppered, graduated cylinder with 20 cc of absolute alcohol, and add 3 portions of 10 cc each of anhydrous ether, shaking thoroly after each addition. Let stand until clear, pour off thru a filter, and wash the cylinder and filter with a mixture of 2 parts of absolute alcohol to 3 parts of anhydrous ether, also pouring the wash liquor thru the filter. Evaporate the filtrate to a sirupy consistency, dry for an hour at the temp. of boiling  $H_2O$ , weigh, ignite, and weigh again. The loss on ignition = the weight of glycerol.

6

### II. By Oxidation with Dichromate—Official

Evaporate 100 cc of the wine in a porcelain dish on a water bath, maintained at a temp. of 85–90°, to a volume of 10 cc. Treat the residue with about 5 g of fine sand and 5 cc of milk of lime (containing 15 g of  $CaO$  per 100 cc). Proceed as directed under **XXXIII, 72**, beginning with the clause "evaporate almost to dryness, with frequent stirring," except to dilute the soln of glycerol after treatment with  $Ag_2CO_3$  and  $Pb$  acetate to a volume of 100 cc instead of 50 cc. Observe the precautions given concerning the temp. at which all evaporations are to be made.

7

### GLYCEROL IN SWEET WINES—OFFICIAL

With wines in which the extract exceeds 5 g per 100 cc, heat 100 cc to boiling in a flask and treat with successive small portions of milk of lime until the wine becomes first darker and then lighter in color. Cool, add 200 cc of 95% alcohol, allow the precipitate to subside, filter, and wash with 95% alcohol. Treat the combined filtrate and washings as directed under 5 or 6.

8

### GLYCEROL-ALCOHOL RATIO—OFFICIAL

Express this ratio as  $X:100$ , in which  $X$  is obtained by multiplying the percentage weight of glycerol by 100 and dividing the result by the percentage of alcohol by weight.

### EXTRACT

9

### I. From the Specific Gravity of the Dealcoholized Wine—Official

Calculate the sp. gr. of the dealcoholized wine,  $D$ , by the following formula:

$$D = S + 1 - A.$$

$S$  = sp. gr. of the sample, 3;

$A$  = sp. gr. of the alcoholic distillate, 4 (a).

From Table 2, under **XLII**, ascertain the percentage by weight of extract in the dealcoholized wine corresponding to the value of  $D$ . The figure thus obtained  $\times$  the value of  $S$  = the g of extract per 100 cc of wine.

10

### II. By Evaporation—Official

(a) *In dry wines, extract content of less than 3 g per 100 cc.*—In a 75 cc flat-bottomed Pt dish, approximately 85 mm in diameter, evaporate 50 cc of the sample on a water bath to a sirupy consistency. Heat the residue for 2–5 hours in a drying oven at the temp. of boiling  $H_2O$ , cool in a desiccator, and weigh as soon as the dish and contents reach room temp.

(b) *In sweet wines.*—If the extract content is 3–6 g per 100 cc, treat 25 cc of the sample as directed under (a). If the extract exceeds 6 g per 100 cc, however, accept

the result obtained as directed under 9, and attempt no gravimetric determination because of the inaccurate results obtained by drying levulose at a high temp.

**11 NON-SUGAR SOLIDS (SUGAR-FREE EXTRACT)—OFFICIAL**

Subtract the quantity of reducing sugars before inversion, 12, from the extract, 9 or 10. If sucrose is present, determine the non-sugar solids by subtracting the sum of reducing sugars before inversion and the sucrose from the extract.

**12 REDUCING SUGARS—OFFICIAL**

(a) *Dry wines*.—Place 200 cc of the sample in a porcelain dish, exactly neutralize with normal NaOH, calculating the quantity required from the determination of acidity, and evaporate to about  $\frac{1}{2}$  the original volume. Transfer to a 200 cc flask, add sufficient neutral Pb acetate soln to clarify, dilute to the mark with H<sub>2</sub>O, shake, and pass thru a folded filter. Remove the Pb with dry K oxalate and determine reducing sugars as directed under XXXIV, 37.

(b) *Sweet wines*.—Approximate the sugar content by subtracting 2 from the result in the determination of the extract and use such a quantity of the sample that the aliquot taken for the Cu reduction shall not exceed 240 mg of invert sugar. Proceed as directed under (a).

**SUCROSE**

**13 I. By Reducing Sugars Before and After Inversion—Official**

Proceed as directed under XXXIV, 28, using the method given under XXXIV, 37 for the determination of reducing sugars.

**14 II. By Polarization—Official**

Polarize before and after inversion in a 200 mm tube, as directed under XXXIV, 22 or 23, a portion of the filtrate obtained under 12. In calculating the percentage of sucrose do not fail to take into consideration the relation of the weight of the sample contained in 100 cc to the normal weight for the instrument.

**15 COMMERCIAL GLUCOSE—OFFICIAL**

Polarize a portion of the filtrate obtained under 12, after inversion in a 200 mm jacketed tube at 87°, as directed under XXXIV, 29. In calculating the percentage of glucose do not fail to take into consideration the relation of the weight of the sample contained in 100 cc to the normal weight for the instrument.

**16 ASH—OFFICIAL**

Proceed as directed under XXVII, 8, using the residue from 50 cc of the wine. Char carefully (decrepitation), and do not exceed 550° during ashing.

**17 ALKALINITY OF THE WATER-SOLUBLE ASH—OFFICIAL**

Extract the ash obtained as directed under 16 with successive small portions of hot H<sub>2</sub>O until the filtrate amounts to about 60 cc and proceed as directed under XXXIV, 13. Express the alkalinity in terms of the number of cc of 0.1 N acid required to neutralize the H<sub>2</sub>O-soluble ash from 100 cc of the wine.

**18 ALKALINITY OF THE WATER-INSOLUBLE ASH—OFFICIAL**

Ignite the filter and residue from 17 in the Pt dish in which the wine was ashed and proceed as directed under XXXIV, 14. Express the alkalinity in terms of the number of cc of 0.1 N acid required to neutralize the water-insoluble ash from 100 cc of the wine.

## 19

## PHOSPHORIC ACID—OFFICIAL

Dissolve the ash, 16, in 50 cc of boiling  $\text{HNO}_3$  (1+9), filter, wash the paper, and determine  $\text{P}_2\text{O}_5$  in the combined filtrate and washings as directed under II, 9 or 12. If the ash ignites without difficulty, no free phosphoric acid need be suspected. If any free acid is present, the ash remains black even after repeated leaching. In the latter case, add Ca acetate or a mixture containing 3 parts of  $\text{Na}_2\text{CO}_3$  and 1 part of  $\text{NaNO}_3$  to avoid loss of  $\text{P}_2\text{O}_5$  before attempting to ash.

## 20

## SULFURIC ACID—OFFICIAL

Precipitate directly the  $\text{H}_2\text{SO}_4$  in 50 cc of the sample by means of 10%  $\text{BaCl}_2$  soln after acidifying with a small excess of  $\text{HCl}$ , and determine the resulting  $\text{BaSO}_4$  as directed under XII, 27. Allow the precipitate to stand for at least 6 hours before filtering. Report as  $\text{SO}_3$ , using the factor 0.3430.

## 21

## CHLORIDES—OFFICIAL

To 100 cc of dry wine or 50 cc of sweet wine, add sufficient  $\text{Na}_2\text{CO}_3$  to make distinctly alkaline. Evaporate to dryness, ignite at a heat not above low redness, cool, extract the residue with hot  $\text{H}_2\text{O}$ , acidify the  $\text{H}_2\text{O}$  extract with  $\text{HNO}_3$  (1+4), and determine chlorides as directed under XII, 35 or 37.

## 22

## ACIDITY—OFFICIAL

(a) *Phenolphthalein*.—In a large porcelain dish neutralize about 250 cc of recently boiled  $\text{H}_2\text{O}$  with 0.1 N alkali, using about 2 cc of phenolphthalein indicator soln. Add the wine and titrate rapidly to a distinct pink. Heat the portion of wine to be titrated to incipient boiling to remove  $\text{CO}_2$  (all wines, still or gaseous) and transfer it to the dish with a portion of the neutralized  $\text{H}_2\text{O}$ . The quantity to be used depends on the depth of color of the wine; it is generally 5 cc for deeply colored red wine and 20 cc for white wine.

(b) *Azolitmin*.—Measure 20 cc of the wine into a 250 cc beaker, heat rapidly to incipient boiling, and immediately titrate with 0.1 N  $\text{NaOH}$  soln. Determine the end point with neutral 0.05% azolitmin soln as an outside indicator. Place the indicator in the cavities of a spot plate and spot the wine into the azolitmin soln. The end point is reached when the color of the indicator remains unchanged by the addition to the wine of a few drops of 0.1 N alkali.

(c) *Phenolphthalein powder* (artificially colored wines).—Into the cavities of a spot plate place a mixture of one part of phenolphthalein and 100 parts of dry powdered  $\text{K}_2\text{SO}_4$  and spot the wine into the powder. (The end of the titration is indicated when the powder acquires a pink tint. The powder should not be too fine. The addition of neutral alcohol to the wine will facilitate the flow of the wine into the powder.) Tilt the spot plate and allow the wine to flow into the powder from the tip of a heavy stirring rod.

Express the results in terms of tartaric acid. 1 cc of 0.1 N  $\text{NaOH}$  soln = 0.0075 g of tartaric acid.

## TOTAL VOLATILE ACIDITY

## 23

## Method I—Official

Heat rapidly to incipient boiling 50 cc of the wine in a 500 cc distillation flask and pass steam thru until 15 cc of the distillate requires only 2 drops of 0.1 N  $\text{NaOH}$  soln for neutralization. Boil the  $\text{H}_2\text{O}$  used to generate the steam several minutes before connecting the steam generator with the distillation flask in order to expel

CO<sub>2</sub>. Titrate rapidly with 0.1 *N* NaOH soln, using phenolphthalein indicator. (The color should remain about 10 seconds.) Express results as acetic acid. 1 cc of 0.1 *N* NaOH soln = 0.0060 g of acetic acid.

24

*Method II—Official*

(Preferred for wines containing an abnormal quantity of acetic acid.)

Introduce 10 cc of the wine, previously freed from CO<sub>2</sub> by heating to incipient boiling, into the inner tube of a modified Sellier distillation apparatus (Fig. 17); add a small piece of paraffin to prevent foaming, and adjust the tube and its contents in place within the larger flask, which contains 100 cc of recently boiled H<sub>2</sub>O.

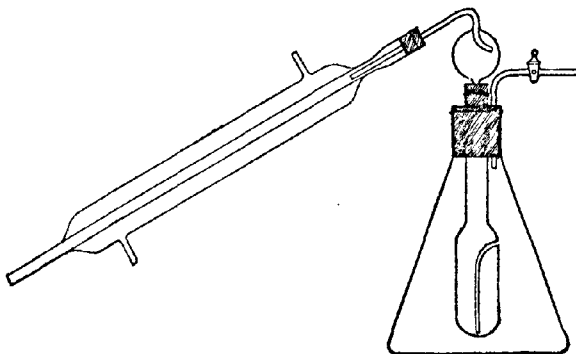


FIG. 17.—APPARATUS FOR THE DETERMINATION OF VOLATILE ACIDITY

Connect with a condenser as illustrated in the figure and distil by heating the outer flask. When 50 cc of the distillate has been collected, empty the receiver into a beaker and titrate with 0.1 *N* NaOH soln, using phenolphthalein indicator. Continue the distillation and titrate each succeeding 10 cc of distillate until not more than 1 drop of standard alkali is required to reach the neutral point. Usually 80 cc of distillate will contain all the volatile acids.

25

**TOTAL ACIDITY—EXCLUSIVE OF SO<sub>2</sub>**

As directed under XXXII, 33, determine SO<sub>2</sub> in the distillate obtained under 23 or 24. Calculate in terms of acetic acid and subtract from the total volatile acidity.

26

**FIXED ACIDITY—OFFICIAL**

Calculate the fixed acidity as tartaric by multiplying the total volatile acidity by 1.25 and subtracting this product from the total acidity.

27

**TOTAL TARTARIC ACID?—OFFICIAL**

Neutralize 100 cc of the sample with *N* NaOH soln, calculating from the acidity, 22, the number of cc of *N* alkali necessary. If the volume of the soln is increased more than 10% by the addition of the alkali, evaporate to approximately 100 cc. Add to the neutralized soln 0.075 g of tartaric acid for each cc of *N* alkali added. It is essential that the tartaric acid be pure; if necessary recrystallize. After the tartaric acid has dissolved add 2 cc of glacial acetic acid and 15 g of KCl. After the KCl has dissolved, add 15 cc of 95% alcohol, stir vigorously until the K bitartrate

begins to precipitate, and let stand in an ice box at 15–18° for at least 15 hours. Decant the liquid from the separated K bitartrate on a Gooch crucible prepared with a very thin film of asbestos, or on filter paper in a Büchner funnel. Wash the precipitate from the beaker with the filtrate (keep cold) and finally rinse the beaker and filter 3 times with a few cc of a mixture of 15 g of KCl, 20 cc of 95% alcohol, and 100 cc of H<sub>2</sub>O, using not more than 20 cc of the wash soln in all. Transfer the asbestos or paper and precipitate to the beaker in which the precipitation was made; wash the Gooch crucible or Büchner funnel with hot H<sub>2</sub>O, using about 50 cc in all; heat to boiling and titrate the hot soln with 0.1 N NaOH soln, using phenolphthalein indicator. Increase the number of cc of 0.1 N alkali required by 1.5 cc to allow for the solubility of the precipitate. Under these conditions 1 cc of 0.1 N alkali = 0.015 g of tartaric acid. To obtain the g of total tartaric acid per 100 cc of the wine, subtract the quantity of tartaric acid added from this result.

## 28 FREE TARTARIC ACID AND CREAM OF TARTAR<sup>3</sup>—OFFICIAL

Calculate in the following manner:

*A* = total tartaric acid, 27, divided by 0.015;

*B* = total alkalinity of the ash (*C* + *D*);

*C* = alkalinity of water-soluble ash, 17; and

*D* = alkalinity of water-insoluble ash, 18.

Then

- (1) If *A* is greater than *B*,  
     Cream of tartar =  $0.0188 \times C$ , and  
     Free tartaric acid =  $0.015 \times (A - B)$ ;
- (2) If *A* equals *B* or is smaller than *B* but greater than *C*,  
     Cream of tartar =  $0.0188 \times C$ , and  
     Free tartaric acid = 0; and
- (3) If *A* is smaller than *C*,  
     Cream of tartar =  $0.0188 \times A$ , and  
     Free tartaric acid = 0.

## 29 CITRIC AND MALIC ACIDS—TENTATIVE

For citric and malic acids occurring in normal wines in small quantities only, use 100 cc of sample and evaporate to 45 cc. After saponification proceed as directed under XXVI.

## TANNIN AND COLORING MATTER—OFFICIAL

### 30

#### REAGENTS

- (a) *Oxalic acid soln.*—0.1 N. 1 cc = 0.00416 g of tannin.
- (b) *Standard potassium permanganate soln.*—Dissolve 1.333 g of KMnO<sub>4</sub> in 1 liter of H<sub>2</sub>O and standardize the soln against (a).
- (c) *Indigo soln.*—Dissolve 6 g of Na indigotin sulfonate in 500 cc of H<sub>2</sub>O by heating; cool, add 50 cc of H<sub>2</sub>SO<sub>4</sub>, make up to 1 liter, and filter.
- (d) *Purified boneblack.*—Boil 100 g of finely powdered boneblack with successive portions of HCl (1 + 3), filter, and wash with boiling H<sub>2</sub>O until free from chlorides. Keep covered with H<sub>2</sub>O.

### 31

#### DETERMINATION<sup>4</sup>

Dealccoholize 100 cc of the wine by evaporation and dilute with H<sub>2</sub>O to the original volume. Transfer 10 cc to a 2 liter porcelain dish and add about 1 liter of H<sub>2</sub>O and exactly 20 cc of the indigo soln. Add the standard KMnO<sub>4</sub> soln, 1 cc at a time, until the blue color changes to green; then add a few drops at a time until the

color becomes golden yellow. Designate the number of cc of  $\text{KMnO}_4$  soln as "a."

Treat 10 cc of the prepared dealcoholized wine for 15 min. with boneblack, filter, and wash thoroly with  $\text{H}_2\text{O}$ . Add 1 liter of  $\text{H}_2\text{O}$  and 20 cc of the indigo soln and titrate with  $\text{KMnO}_4$ , as directed above. Designate the number of cc of  $\text{KMnO}_4$  used as "b."

Then  $a - b = c$ , the number of cc of the  $\text{KMnO}_4$  soln required for the oxidation of the tannin and coloring matter in 10 cc of the wine.

32

#### CRUDE PROTEIN—OFFICIAL

Determine N in 50 cc of the wine as directed under II, 21, 23, or 25, and multiply the result by 6.25.

33

#### PENTOSANS—OFFICIAL

(Applicable to dry wines only.)

Proceed as directed under XXVII, 36, except to use 100 cc of the wine and 43 cc of  $\text{HCl}$  in beginning the distillation. (Owing to the interference of sugars this determination can be made in dry wines only.)

34

#### GUM AND DEXTRIN—TENTATIVE

Evaporate 100 cc of the wine to about 10 cc and add 10 cc of 95% alcohol. If gum or dextrin is present (indicated by the formation of a voluminous precipitate), continue the addition of alcohol, slowly and with stirring, until 100 cc has been added. Let stand overnight, filter, and wash with alcohol, 80% by volume. Dissolve the precipitate on the paper with hot  $\text{H}_2\text{O}$ , hydrolyze the filtrate and washings with  $\text{HCl}$ , and proceed as directed under XXVII, 31.

35

#### NITRATES—TENTATIVE

(a) *White wine*.—Treat a few drops of the wine in a porcelain dish with 2-3 cc of  $\text{H}_2\text{SO}_4$  that contains about 0.1 g of diphenylamine<sup>1</sup> per 100 cc. The deep blue color formed in the presence of nitrates appears so quickly that it is not obscured, even in sweet wine, by the blackening produced by the action of  $\text{H}_2\text{SO}_4$  on the sugar.

(b) *Red wine*.—Clarify with basic Pb acetate, filter, remove the excess of Pb from the filtrate with  $\text{Na}_2\text{SO}_4$ , filter again, and treat a few drops of this filtrate as directed under (a).

36

#### COLORING MATTERS—TENTATIVE

Proceed as directed under XXI.

37

#### PRESERVATIVES—OFFICIAL

Proceed as directed under XXXII. The determination of  $\text{SO}_2$  should be quantitative. The occurrence of small quantities of salicylic acid in the grape has been reported in the literature and for that reason not more than 50 cc of sample should be used in testing for that preservative. Concord grapes contain 3.2 mg of salicylic acid per 100 cc of juice.<sup>6</sup>

#### SELECTED REFERENCES

- <sup>1</sup> J. Ind. Eng. Chem., **1**, 31 (1909).
- <sup>2</sup> U. S. Dept. Agr. Bur. Chem. Bull. **162**, p. 72.
- <sup>3</sup> Ibid., p. 75.
- <sup>4</sup> Ann. Oenologie, **2**, 1 (1871-72).
- <sup>5</sup> Arch. Hyg., **2**, 273 (1884).
- <sup>6</sup> J. Am. Chem. Soc., **25**, 242 (1903)

## XVI. DISTILLED LIQUORS

### SPIRITS

#### 1 PHYSICAL EXAMINATION—TENTATIVE

Note and record the following: (1) color and depth of color; (2) odor—whiskey, brandy, rum, etc., or foreign; (3) taste—whiskey, brandy, rum, etc., or foreign.

#### 2 SPECIFIC GRAVITY—OFFICIAL

Determine the specific gravity at 20/20° by means of a pycnometer, as directed under XIV, 3, or by means of a small, accurately graduated hydrometer.

#### 3 ALCOHOL BY WEIGHT—OFFICIAL

Weigh 20–25 g of the sample into a distillation flask, dilute with 100 cc of H<sub>2</sub>O, and distil nearly 100 cc. Weigh the distillate or make to volume at room temp., noting the temp. In either case determine the sp. gr. as directed under XIV, 3, at room temp. if desired. Obtain the corresponding percentage of alcohol by weight from Tables 19 and 21, XLII, multiply this figure by the weight of the distillate, and divide by the weight of the sample taken.

The alcohol content of the distillate may be checked by determining the immersion refractometer reading and obtaining the percentage of alcohol from Table 20, under XLII.

#### ALCOHOL BY VOLUME

##### 4 *Method I—Official*

From the sp. gr. of the distillate obtained under 3 ascertain the corresponding percentage of alcohol by volume from XLII, Table 19. Multiply this figure by the volume of distillate and divide by the volume of the sample (calculated from the sp. gr.) to obtain the percentage of alcohol by volume in the original sample.

##### 5 *Method II—Official*

Measure 25 cc of the sample into a distillation flask, noting the temp., dilute with 100 cc of H<sub>2</sub>O, distil nearly 100 cc, make to volume at the same temp., and determine the sp. gr. as directed under XIV, 3. Obtain, from Table 19, XLII, the corresponding percentage of alcohol by volume in the distillate and multiply by 4 to obtain the percentage of alcohol by volume in the original substance.

The alcohol content of the distillate may be checked by determining the immersion refractometer reading and obtaining the percentage of alcohol from Table 20, XLII.

#### 6 EXTRACT—OFFICIAL

Weigh, or measure at 20°, 100 cc of the sample, evaporate nearly to dryness on a steam bath, transfer to a water oven, and dry at the temp. of boiling H<sub>2</sub>O for 2.5 hours.

#### 7 ASH—OFFICIAL

Proceed as directed under XXVII, 8, using the residue from the determination of the extract, 6.

#### 8 ACIDITY—OFFICIAL

Titrate 100 cc of the sample (or 50 cc diluted to 100 cc if the sample is dark) with 0.1 N alkali, using phenolphthalein indicator. Express the result as acetic acid. 1 cc of 0.1 N alkali = 0.0060 g of acetic acid.

9

## ESTERS—OFFICIAL

Measure 100–200 cc of the sample into a distillation flask, add 12.5–25 cc of  $H_2O$ , and distil slowly 100–200 cc, depending upon the amount of sample taken, using a mercury valve to prevent loss of alcohol. Exactly neutralize the free acid in 50 cc of the distillate with 0.1 *N* alkali and add a measured excess of 25–50 cc of 0.1 *N* alkali. Then either boil for an hour under a reflux condenser, cool, and titrate with 0.1 *N* acid, or allow the soln to stand overnight in a stoppered flask with the excess of alkali, heat with a tube condenser for 30 min. at a temp. below the boiling point, cool, and titrate. Calculate the number of cc of 0.1 *N* alkali used in the saponification of the esters as ethyl acetate. 1 cc of 0.1 *N* alkali = 0.0088 g of ethyl acetate. Run a blank, using  $H_2O$  in place of the distillate, and make any necessary correction.

## ALDEHYDES—OFFICIAL

10

## REAGENTS

(a) *Aldehyde-free alcohol*.—Redistil 95% alcohol over NaOH or KOH, add 2–3 g per liter of metaphenylenediamine hydrochloride, digest at ordinary temp. for several days (or under a reflux condenser on a steam bath for several hours), and distil slowly, rejecting the first 100 cc and the last 200 cc of the distillate.

(b) *Sulfite-fuchsin soln*.—Dissolve 0.50 g of pure fuchsin in 500 cc of  $H_2O$ , add 5 g of  $SO_2$  dissolved in  $H_2O$ , make up to 1 liter, and allow to stand until colorless. As this soln decomposes rapidly, prepare it in small quantities and keep at a low temp.

(c) *Standard acetaldehyde soln*.—Prepare according to the directions of Vasey,<sup>1</sup> as follows: Grind aldehyde ammonia in a mortar with anhydrous ether and decant the ether. Repeat this operation several times and dry the purified salt in a current of air and then in vacuo over  $H_2SO_4$ . Dissolve 1.386 g of this purified aldehyde ammonia in 50 cc of 95% alcohol, add 22.7 cc of *N* alcoholic  $H_2SO_4$ , make up to 100 cc and add 0.8 cc of alcohol for the volume of the  $(NH_4)_2SO_4$  precipitate. Allow the mixture to stand overnight, and filter. This soln contains 1 g of acetaldehyde in 100 cc and will retain its strength.

The standard found most convenient for use is 2 cc of this strong aldehyde soln diluted to 100 cc with alcohol, 50% by volume. 1 cc of this soln = 0.0002 g of acetaldehyde. Make up the soln every day or so, as it loses strength.

11

## DETERMINATION

Determine the aldehyde in the prepared distillate, 9. Dilute 5–10 cc of the distillate to 50 cc with aldehyde-free alcohol, 50% by volume; add 25 cc of the sulfite-fuchsin soln, and allow to stand for 15 min. at 15°. The solns and reagents should be at 15° when they are mixed. Prepare standards of known strength and blanks in the same way. The comparison standards found most convenient for use contain 0.1, 0.2, 0.3, 0.4, 0.5, and 0.6 mg of acetaldehyde.

## FURFURAL—OFFICIAL

12

## REAGENT

*Standard furfural soln*.—Dissolve 1 g of redistilled furfural in 100 cc of 95% alcohol. Prepare standards by diluting 1 cc of this soln to 100 cc with alcohol, 50% by volume. 1 cc of this soln contains 0.1 mg of furfural. (The strong furfural soln will retain its strength, but the dilute soln will not.)



## 13

## DETERMINATION

Dilute 10–20 cc of the prepared distillate, 9, to 50 cc with furfural-free alcohol, 50% by volume. Add 2 cc of colorless aniline and 0.5 cc of HCl (sp. gr. 1.125) and keep for 15 min. in a water bath at about 15°. Prepare standards of known strength and blanks in the same way. The comparison standards found most convenient for use contain 0.05, 0.1, 0.15, 0.2, 0.25, and 0.3 mg of furfural.

DETECTION OF ACETONE, KETONES, ISOPROPYL ALCOHOL,  
AND TERTIARY BUTYL ALCOHOL—TENTATIVE

## 14

## REAGENT

*Mercuric sulfate soln.*—Mix 5 g of yellow HgO in 40 cc of H<sub>2</sub>O, add with stirring 20 cc of H<sub>2</sub>SO<sub>4</sub> and 40 cc of H<sub>2</sub>O, and stir until completely dissolved.

## 15

## DETERMINATION

To 2 cc of distillate, 9, add 3 cc of H<sub>2</sub>O and 10 cc of the mercuric sulfate soln. Heat on the boiling water bath for 3 min. A white or yellow precipitate forming within 3 min. indicates the presence of any of the above-mentioned compounds. Any precipitate forming after 3 min. on the boiling water bath should be disregarded.

## FUSEL OIL—OFFICIAL

## 16

## REAGENTS

(a) *Purified carbon tetrachloride.*—Mix in a separatory funnel crude CCl<sub>4</sub> with 1/10 its volume of H<sub>2</sub>SO<sub>4</sub>, shake thoroly at frequent intervals, and allow to stand overnight. Wash free of acid and impurities with tap H<sub>2</sub>O, remove the H<sub>2</sub>O, add an excess of NaOH soln, and distil the CCl<sub>4</sub>.

The refuse CCl<sub>4</sub> after titration is purified for further work by collecting in a large bottle, adding NaOH soln (1+1), shaking, washing with tap H<sub>2</sub>O until the washings are neutral to phenolphthalein, and distilling.

(b) *Oxidizing soln.*—Dissolve 100 g of K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> in 900 cc of H<sub>2</sub>O and add 100 cc of H<sub>2</sub>SO<sub>4</sub>.

## 17

## DETERMINATION

(1) To 50 cc of the sample add 50 cc of H<sub>2</sub>O then 20 cc of 0.5 N NaOH, and saponify the mixture by boiling for an hour under a reflux condenser; or, (2) mix 50 cc of the liquid and 50 cc of H<sub>2</sub>O with 20 cc of 0.5 N NaOH, allow to stand overnight at room temp., and distil directly. Connect the flask with a distillation apparatus, distil 90 cc, add 25 cc of H<sub>2</sub>O, and continue the distillation until an additional 25 cc is collected.

Whenever aldehydes are present in excess of 15 parts per 100,000, add to the distillate 0.5 g of metaphenylenediamine hydrochloride, boil under a reflux condenser for an hour, distil 100 cc, add 25 cc of H<sub>2</sub>O, and continue the distillation until an additional 25 cc is collected. Approximately saturate the distillate with finely ground NaCl and add saturated NaCl soln until the sp. gr. is 1.10. Extract this salt soln 4 times with the purified CCl<sub>4</sub>, using 40, 30, 20, and 10 cc, respectively, and wash the CCl<sub>4</sub> 3 times with 50 cc portions of saturated NaCl soln, and twice with

saturated  $\text{Na}_2\text{SO}_4$  soln. Transfer the  $\text{CCl}_4$  to a flask containing 50 cc of the oxidizing soln and boil for 8 hours under a reflux condenser.

Add 100 cc of  $\text{H}_2\text{O}$  and distil until only about 50 cc remains. Add 50 cc of  $\text{H}_2\text{O}$  and again distil until 35–50 cc is left. Use extreme care to prevent the oxidizing mixture from burning and baking on the side of the distilling flask. The distillate should be water white; if it is colored discard it and repeat the determination. Titrate the distillate with 0.1 *N*  $\text{NaOH}$ , using phenolphthalein indicator. 1 cc of 0.1 *N*  $\text{NaOH}$  = 0.0088 g of amyl alcohol.

If preferred, use rubber stoppers in the saponification and first distillation, but use corks covered with tinfoil in the oxidation and second distillation. Renew the corks and tinfoil frequently.

Conduct a blank determination upon 100 cc of  $\text{CCl}_4$ , beginning the blank at that point of the procedure immediately after the extraction and just before the washings with  $\text{NaCl}$  and  $\text{Na}_2\text{SO}_4$  solns.

18

## SUGARS—OFFICIAL

Proceed as directed under XXXIV.

## METHYL ALCOHOL

19

*Trillat Method<sup>2</sup>—Official*

To 50 cc of the sample add 50 cc of  $\text{H}_2\text{O}$  and 8 g of lime and fractionate by the aid of Glinsky bulb tubes. Dilute the first 15 cc of the distillate to 150 cc, mix with 15 g of  $\text{K}_2\text{Cr}_2\text{O}_7$  and 70 cc of  $\text{H}_2\text{SO}_4$  (1+5), and allow to stand for 1 hour, shaking occasionally.

Distil, reject the first 25 cc, and collect 100 cc. Mix 50 cc of the distillate with 1 cc of redistilled dimethylaniline, transfer to a stout tightly stoppered flask, and keep on a bath at 70–80° for 3 hours, shaking occasionally. Make distinctly alkaline with  $\text{NaOH}$  soln and distil off the excess of dimethylaniline, stopping the distillation when 25 cc has passed over.

Acidify the residue in the flask with acetic acid, shake, and test a few cc by adding 4 or 5 drops of a 1% suspension of  $\text{PbO}_2$ . If methyl alcohol is present, there occurs a blue coloration, which is increased by boiling. Ethyl alcohol thus treated yields a blue coloration, which changes immediately to green, later to yellow, and becomes colorless when boiled.

20

*Riche and Bardy Method<sup>3</sup>—Official*

The following method depends on the formation of methylaniline violet:

Place 10 cc of the sample, previously redistilled over  $\text{K}_2\text{CO}_3$  if necessary, in a small flask with 15 g of *I* and 2 g of red *P*. Keep in ice  $\text{H}_2\text{O}$  for 10–15 min. or until action has ceased. Distil on a water bath into about 30 cc of  $\text{H}_2\text{O}$ , the methyl and ethyl iodides formed. Wash with dilute alkali to eliminate free *I*. Separate the heavy, oily liquid that settles and transfer to a flask containing 5 cc of aniline. If the action is too violent, place the flask in cold  $\text{H}_2\text{O}$ ; if too slow, stimulate by gently warming the flask. After an hour boil the product with  $\text{H}_2\text{O}$ , cool, and add about 20 cc of 15%  $\text{NaOH}$  soln; when the bases rise to the top as an oily layer, fill the flask up to the neck with  $\text{H}_2\text{O}$  and draw them off with a pipet. Oxidize 1 cc of the oily liquid by adding 10 g of a mixture of 100 parts of clean sand, 2 of  $\text{NaCl}$ , and 3

of  $\text{Cu}(\text{NO}_3)_2$ ; mix thoroly; transfer to a glass tube; and heat to  $90^\circ$  for 8–10 hours. Exhaust the product with warm alcohol, filter, and dilute to 100 cc with alcohol. If the sample is free from methyl alcohol, the liquid has a red tint, but in the presence of 1% of methyl alcohol it has a distinct violet shade; with 2.5% the shade is very distinct and still more so with 5%. To detect more minute quantities of methyl alcohol, dilute 5 cc of the colored liquid to 100 cc with  $\text{H}_2\text{O}$  and dilute 5 cc of this again to 400 cc. Heat the liquid thus obtained in a porcelain dish and immerse in it a fragment of white merino (free from S) for 30 min. If the alcohol is pure, the wool will remain white, but if methyl alcohol is present the fiber will become violet, the depth of tint giving a fairly approximate indication of the proportion of methyl alcohol.

*Modified Denigès Method<sup>4</sup>—Tentative*

## 21

## REAGENTS

(a) *Potassium permanganate soln.*—Dissolve 3 g of  $\text{KMnO}_4$  in 15 cc of 85%  $\text{H}_3\text{PO}_4$  and make up to 100 cc with  $\text{H}_2\text{O}$ .

(b) *Oxalic acid soln.*—Dissolve 5 g of oxalic acid in 100 cc of  $\text{H}_2\text{SO}_4$  (1+1).

(c) *Schiff's reagent.*—Dissolve 0.2 g of rosaniline hydrochloride in 120 cc of hot  $\text{H}_2\text{O}$ , cool, add 2 g of anhydrous  $\text{Na}_2\text{SO}_3$  dissolved in 20 cc of  $\text{H}_2\text{O}$ , and 2 cc of  $\text{HCl}$ , make up to 200 cc, and store in well-filled glass-stoppered amber bottles.

## 22

## PROCEDURE

Dilute the alcoholic beverage to 5% total alcohol by volume. Transfer 5 cc of this soln to a 6-in. test tube, add 2 cc of the  $\text{KMnO}_4$  soln, and let stand 10 min. Remove the excess of  $\text{KMnO}_4$  by the addition of 2 cc of the oxalic acid soln. As soon as the  $\text{KMnO}_4$  is decolorized add 5 cc of Schiff's reagent. Mix thoroly and let stand 10 min. If  $\text{HCHO}$  is present, the characteristic reddish purple color is produced.

Run blanks on pure ethyl alcohol and on ethyl alcohol containing about 1% of methanol.

## 23

*Immersion Refractometer Method<sup>5</sup>—Official*

Determine the Zeiss immersion refractometer reading at  $17.5^\circ$  of the distillate obtained in the determination of alcohol. If, on reference to the table, 24, the refractometer reading shows a sp. gr. agreeing with that obtained in the alcohol determination, it may be assumed that no methyl alcohol is present. If, however, there is present an appreciable quantity of methyl alcohol, the low reading will at once indicate the fact. If the absence from the soln of refractive substances other than  $\text{H}_2\text{O}$  and the alcohols is assured, this difference in refraction is conclusive of the presence of methyl alcohol.

The addition of methyl alcohol to ethyl alcohol decreases the refractive index in direct proportion to the quantity added; hence the quantitative calculation is made by interpolation in the table, 24, of the figures for pure ethyl and methyl alcohol of the same sp. gr. as the sample.

*Example.*—The distillate has a sp. gr. at  $15.56^\circ$  of 0.9625 and a refractometer reading at  $17.5^\circ$  of 43.1. By interpolation in the table, the readings for ethyl and methyl alcohol of this gravity are 65.2 and 31.7, respectively, and the difference is 33.5;  $65.2 - 43.1 = 22.1$ ;  $(22.1 + 33.5)100 = 66.0$ , showing that 66.0% of the total alcohol present is methyl alcohol.

24

Scale readings on the Zeiss immersion refractometer at 17.5°,  
corresponding specific gravities of ethyl and methyl alcohol solutions

SP. GR. 15.56°	SCALE READINGS		DIFFER- ENCES	SP. GR. 15.56°	SCALE READINGS		DIFFER- ENCES
	ETHYL ALCOHOL	METHYL ALCOHOL			ETHYL ALCOHOL	METHYL ALCOHOL	
1.0000	15.0	15.0	0.0	.9720	51.5	27.0	24.5
.9990	15.8	15.3	.5	.9710	53.0	27.5	25.5
.9980	16.6	15.6	1.0	.9700	54.6	28.1	26.5
.9970	17.5	15.9	1.6	.9690	56.1	28.7	27.4
.9960	18.5	16.2	2.3	.9680	57.6	29.2	28.4
.9950	19.4	16.5	2.9	.9670	59.1	29.6	29.5
.9940	20.4	16.9	3.5	.9660	60.6	30.1	30.5
.9930	21.4	17.2	4.2	.9650	62.0	30.6	31.4
.9920	22.5	17.5	5.0	.9640	63.3	31.0	32.3
.9910	23.6	17.9	5.7	.9630	64.6	31.5	33.1
.9900	24.7	18.2	6.5	.9620	65.8	31.9	33.9
.9890	25.9	18.6	7.3	.9610	67.0	32.4	34.6
.9880	27.1	19.0	8.1	.9600	68.1	32.8	35.3
.9870	28.4	19.5	8.9	.9590	69.2	33.3	35.9
.9860	29.6	19.9	9.7	.9580	70.2	33.7	36.5
.9850	31.0	20.4	10.6	.9570	71.2	34.1	37.1
.9840	32.4	20.8	11.6	.9560	72.1	34.5	37.6
.9830	33.8	21.3	12.5	.9550	73.0	34.9	38.1
.9820	35.2	21.8	13.4	.9540	73.8	35.3	38.5
.9810	36.7	22.3	14.4	.9530	74.6	35.6	39.0
.9800	38.3	22.8	15.5	.9520	75.4	35.9	39.5
.9790	39.9	23.4	16.5	.9510	76.2	36.2	40.0
.9780	41.5	24.0	17.5	.9500	76.9	36.5	40.4
.9770	43.1	24.5	18.6	.9490	77.6	36.8	40.8
.9760	44.8	25.0	19.8	.9480	78.3	37.0	41.3
.9750	46.5	25.5	21.0	.9470	79.0	37.3	41.7
.9740	48.2	26.0	22.2	.9460	79.7	37.6	42.1
.9730	49.8	26.5	23.3				

The scale readings are applicable only to instruments calibrated in the arbitrary scale units proposed by Pulfrich, *Z. angew. Chem.*, 1899, p. 1168. According to this scale, 14.5 = 1.33300, 50.0 = 1.34650, and 100.0 = 1.36464. If the instrument used is calibrated in other arbitrary units, the refractive index corresponding to the observed reading can be converted into the equivalent Zeiss reading by referring to Table 24.

### Quantitative Methods—Tentative

25

#### PREPARATION OF SAMPLE

Transfer to a distilling flask a quantity of sample that contains 20–25 cc of absolute alcohol and distil slowly, collecting the distillate in a 50 cc volumetric flask. When nearly to the mark, disconnect the receiver and adjust to the mark at room temp. with H<sub>2</sub>O. Determine methyl alcohol as directed under **28**, or under **XXXIX**, 103.

26

#### APPARATUS

Connect by a stopcock (C), a reaction flask (A) with a bulb holding about 50 cc. and a side inlet tube (B) to a reservoir (D) of about 25 cc capacity. The reaction flask also has a second side tube (E) thru which CO<sub>2</sub> is conducted to help carry over the iodides into the receiving flask (F) and thru the condenser (G), which is surrounded by a 12 in. water jacket (H). Maintain the H<sub>2</sub>O in the jacket at 50–55°

by means of  $\text{H}_2\text{O}$  in the flask (I) heated by the burner (J). Surround the reaction flask by the bath (K), which contains ice and  $\text{H}_2\text{O}$  at the beginning of the operation and is later heated by a flame and maintained at  $75\text{--}80^\circ$  during the remainder of the determination. By means of a ground-glass joint connect the outlet tube of the condenser to the tube M, which extends to the bottom of the receiving flask thru one hole of a two-holed rubber stopper. Have the tube N pass thru the second hole of the same stopper and from thence to an empty 50 cc Erlenmeyer flask (O), from which a second tube (P) passes below the surface of the dilute  $\text{H}_2\text{SO}_4$  contained in the second 50 cc Erlenmeyer flask (Q), which is also fitted with an outlet tube (R) leading to the surrounding atmosphere. Convey the overflow from the condenser to the beaker (S) thru the tube (T), passing thru the stopper (U), which also holds the thermometer (V). Keep the receiving flask (125 cc Erlenmeyer) cold by immersing it in the bath (W) containing ice and  $\text{H}_2\text{O}$ .

To collect the precipitate, use sintered glass filtering crucibles similar to Jena No. 1 G 4.

## 27

## REAGENTS

(a) *Trimethylamine soln.*—Cool 100 g of anhydrous trimethylamine in the sealed container below the boiling point ( $+3^\circ$ ) with ice and salt or by placing overnight in a cold room maintained at a temp. below freezing. Similarly, cool about 1 liter of absolute alcohol and a 1 liter graduated flask. Transfer about 700 cc of the absolute alcohol to the flask, open the container of the trimethylamine, and transfer the liquid to the flask, washing out the vessel with small portions of the cold absolute alcohol. Fill the flask to within 50–75 cc of the mark with the alcohol, mix, warm gradually to the temp. of the laboratory, fill to the mark with absolute alcohol, and mix thoroly.

(b) *Wash soln.*—Place about 0.25 g of tetramethylammonium iodide, obtained from a determination of methoxyl, in a 500 cc flask, fill to a convenient height with absolute alcohol, stopper, and shake to saturate the liquid with the salt. Filter the soln as needed thru a white ribbon filter paper.

(c) *Carbon dioxide.*—Obtain from a tank fitted with a reducing valve and a rubber tube connected to the reaction flask.

## 28

## DETERMINATION

Raise the temp. of the  $\text{H}_2\text{O}$  in the condenser jacket (H) to  $50\text{--}55^\circ$  by means of the flame under the flask (I). Place 15 g of 1 and 2 g of red phosphorus in the reaction flask A, attach the flask to the apparatus, and surround it with the bath of ice and  $\text{H}_2\text{O}$  (K). Introduce 2.5 cc of 95% alcohol into the reaction flask thru the reservoir (D). Measure into the reservoir 10–20 cc of the sample, which should contain not more than 0.160 g of methyl alcohol nor more than 7 cc of ethyl alcohol. Place 25 cc of wash soln in the receiving flask (F), connect the flask to the apparatus by means of tube M, and surround it with the bath containing ice and  $\text{H}_2\text{O}$  (W). Attach the  $\text{CO}_2$  tank and so adjust as to make it possible to start the current of gas at a moment's notice. Stir the ice and  $\text{H}_2\text{O}$  in the bath to cool the reaction flask to as near  $0^\circ$  as possible. Now adjust the stopcock (C) so that the sample will flow slowly down the cold sides of the reaction flask (A). (The addition of 10 cc of sample should require 3–5 min.) Stir the ice and  $\text{H}_2\text{O}$  constantly during the addition. When all the sample has run in, wash the sides of the reservoir with two or three small portions of  $\text{H}_2\text{O}$ , using 5–10 cc, and add the washings to the contents of the reaction flask. Fill the reservoir with  $\text{H}_2\text{O}$  to prevent leakage. Remove the ice from the bath,

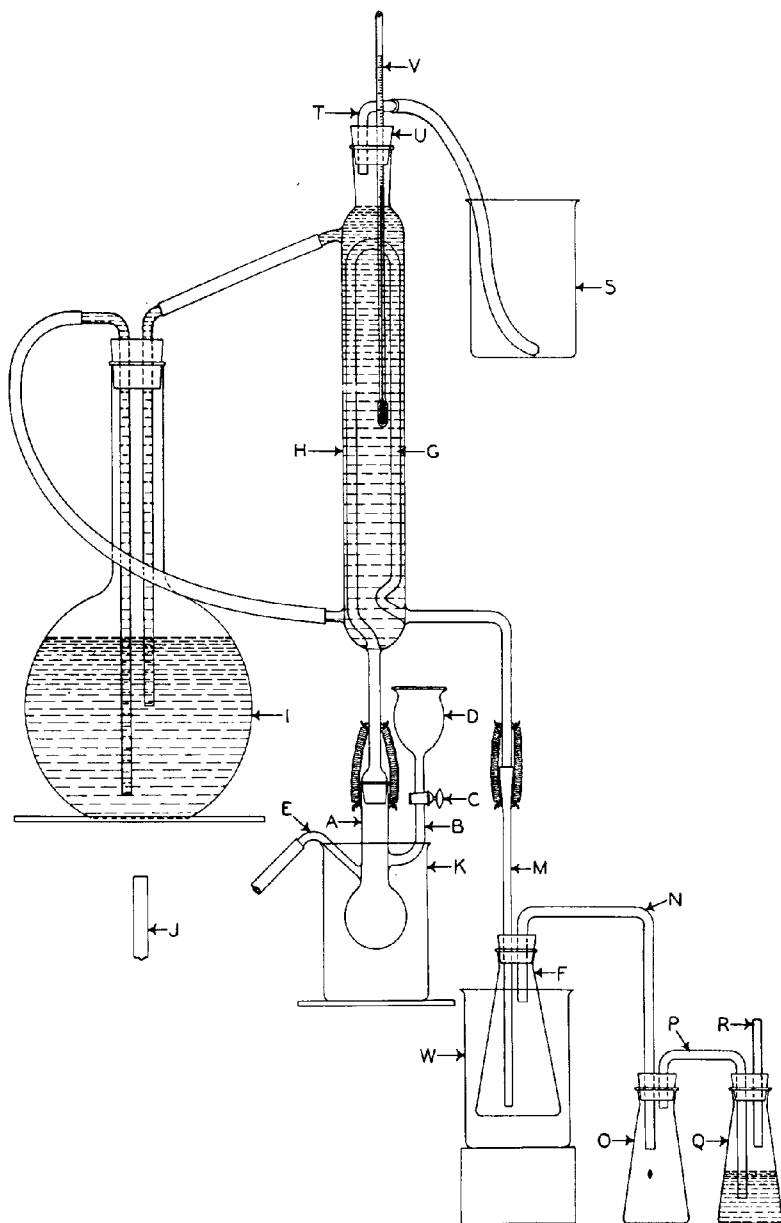


FIG. 18.—APPARATUS FOR THE DETERMINATION OF METHYL ALCOHOL

leaving the cold  $\text{H}_2\text{O}$ . (At this point it will be noticed that the mixture in the reaction flask consists of two layers: the bottom one, dark red; the upper one, colorless or nearly so. The two layers will gradually mingle. If vapors of  $\text{HI}$  are seen to rise from the surface of the liquid, due to violence of the reaction during the mixing of the two layers, cool by stirring the bath, adding a small piece of ice if necessary to prevent the mixture from boiling or giving off acid vapors too rapidly.)

When the contents of the reaction flask have become homogeneous except for the floating particles of phosphorus, place a burner under the bath and heat fairly rapidly to  $75^\circ$ . During the heating add to the receiving flask 10 cc of trimethylamine soln and 25 cc of wash soln, and attach the flask containing the dilute  $\text{H}_2\text{SO}_4$  to prevent the trimethylamine from escaping into the air. When the contents of the reaction flask begin to boil, turn on the  $\text{CO}_2$  at the rate of about 50 bubbles per min., counting them as they arise from the tube in the receiving flask. Allow the distillation to proceed for 1.5–2 hours, maintaining the temp. of the bath at  $75\text{--}80^\circ$ , the jacket (H) at  $50\text{--}55^\circ$ , and the bath (W) at or near  $0^\circ$ .

Disconnect the receiving flask and wash out the tube (M) with 10–15 cc of wash soln, using a rubber policeman to scrub off any crystals that may adhere to the outside of the tube and a small glass rod to remove crystals from the inside, if necessary. Stopper the flask and let stand at room temp. overnight. (The stopper should remain loose until the contents of the flask have reached the temp. of the room.) Filter the mixture on a weighed sintered glass crucible, using 35–40 cc of wash soln to transfer the precipitate from the flask to the crucible. If the filtrate becomes cloudy or crystals separate out, do not be concerned, since the soln may contain large quantities of trimethylethylammonium iodide, which is only soluble to the extent of about 4 g per 100 cc of absolute alcohol, and large quantities of crystals are deposited due to the rapid evaporation of the alcohol in the suction flask. At this point wash off the outside of the crucible with 95% alcohol to remove the crystals of trimethylethylammonium iodide that have formed on the bottom and lower sides of the crucible, and suck dry. Now wash the contents and inside of the crucible three times in the following manner: Turn off the suction, add about 5 cc of wash soln by pouring down the sides of the crucible, and mix the liquid with the crystals by rotating the crucible or by stirring up the contents with a small glass rod or a fine stream of wash soln from a wash bottle. Wash off the rod. Cover the crucible with a small watch-glass and let stand 2–3 min. Suck dry. After the third washing, remove the crucible from the holder, and carefully wash off the outside of the crucible with 95% alcohol, sucking dry at once if any liquid gets on the bottom of the sinter. Dry at  $100\text{--}110^\circ$  for 1 hour, cool in a desiccator, and weigh. The weight of precipitate  $\times$  the factor 0.15933 = the corresponding weight of methyl alcohol in the portion taken for analysis.

In determining 5 mg or less, Pregl filtering tubes may be used to advantage.

## 29

### COLORING MATTERS—TENTATIVE

Proceed as directed under **XXI**, 2 and 8–24, inc.

## 30

### WATER-INSOLUBLE COLOR—TENTATIVE

Evaporate 50 cc of the sample just to dryness on a steam bath. Take up with approximately 15 cc of cold  $\text{H}_2\text{O}$ , filter, and wash until the filtrate amounts to nearly 25 cc. To this filtrate add 25 cc of absolute alcohol, or 26.3 cc of 95% alcohol, and make up to 50 cc with  $\text{H}_2\text{O}$ . Mix thoroly and compare in a colorimeter with the original material. Calculate from these readings the percentage of color insoluble in  $\text{H}_2\text{O}$ .

**31 COLOR INSOLUBLE IN AMYL ALCOHOL—TENTATIVE**

Evaporate 50 cc of the sample just to dryness on a steam bath. Dissolve the residue in  $H_2O$  and 95% alcohol and make to a volume of 50 cc, using a total volume of 26.3 cc of 95% alcohol. Place 25 cc of this soln in a separatory funnel and add 20 cc of freshly shaken Marsh reagent (100 cc of pure amyl alcohol, 3 cc of sirupy  $H_3PO_4$ , and 3 cc of  $H_2O$ ), shaking lightly so as not to form an emulsion. Allow the layers to separate and repeat this shaking and standing twice. After the layers have separated completely draw off the lower or aqueous layer, which contains the caramel, into a 25 cc cylinder and make up to volume with alcohol, 50% by volume. Compare this soln in a colorimeter with the untreated 25 cc. Calculate from this reading the percentage of color insoluble in amyl alcohol.

**ARTIFICIAL COLORS****32***Marsh Test<sup>1</sup>—Tentative*

To 10 cc of the sample in a 20 cc test tube, add sufficient Marsh reagent (31) to nearly fill the tube, and shake several times. Allow the layers to separate. Color in the lower layer indicates that the sample has been colored with caramel, a coal tar dye, or with extractive material from uncharred white oak chips.

In the absence of any color, test 10 cc in the same manner, using sufficient fusel oil, amyl alcohol, or pentasol to nearly fill the tube, and shaking several times. A deeply colored lower layer is an indication of a coal tar dye. Ascertain its identity as directed under **XXI**. To confirm caramel apply the following modified Marsh test.

**33***Modified Marsh Test<sup>2</sup>—Official, First Action*

Place 25 cc of the spirits in a 150 cc beaker marked to show volumes of 13 cc and of 25 cc; add 0.5 cc of glacial acetic acid, 0.75 g of zinc acetate crystals, U.S.P., and mix. When the crystals are nearly dissolved, boil down rapidly over a flame to the 13 cc mark, stirring frequently to prevent bumping or spattering. If the liquid should inadvertently go below the 13 cc mark, fill to that mark with  $H_2O$  and set aside to cool. When cooled to room temp. fill to the 25 cc mark with 95% alcohol, mix, and allow to stand 2–3 min. Mix again, and filter thru a double filter (folded or S. & S. white ribbon). Mix the filtrate and transfer 6 cc to a 6 in. test tube; add 12 cc of Marsh reagent, 31, and mix thoroly until the voluminous white precipitate which forms when the liquids first mix goes back into solution. Allow to stand until the layers separate, then pour off 4 cc of the upper layer into a graduated cylinder and in its place in the test tube pour 4 cc of 88% grade ethyl acetate; mix, and allow to stand until the layers separate. A dark brown color in the lower layer indicates that caramel is present. If, however, the lower layer is colorless and a positive Marsh test was obtained under 32, coloring from uncharred white oak chips is indicated. If the lower layer has a reddish shade, coal tar colors may be present. Confirm the presence of coal tar color by transferring some of the remaining filtrate to a porcelain dish and adding a few drops of HCl. If coal tar colors are present, the soln may become red. For further confirmation add  $SnCl_2$  soln, which will decolorize the soln. Use the clear light of an open window as a background for examining the colors obtained in these tests.

**CORDIALS AND LIQUEURS—TENTATIVE****34****PHYSICAL EXAMINATION**

Note and record the following: (1) Appearance, whether bright or turbid and whether there is any sediment; (2) color and depth of color; (3) odor; (4) taste.



35

## SPECIFIC GRAVITY

Determine sp. gr. at 20/20° as directed in XIV, 3.

36

## ALCOHOL

- (a) *By weight*.—Proceed as directed under 3.  
(b) *By volume*.—Proceed as directed under 4 or 5.

37

## TOTAL SOLIDS

- (a) *From the sp. gr. of the dealcoholized sample*.—Proceed as directed under XIV, 6.  
(b) *By evaporation*.—Proceed as directed under XXXIV, 4.  
(c) *From the residue of the dealcoholized sample*.—Restore the residue from the alcohol determination to its original volume by making the necessary evaporation or dilution. Determine the refractometer reading of the soln at 20° and obtain the corresponding percentage of dry substance. From Table 2, XLII, ascertain the sp. gr. corresponding to the percentage of dry substance found and multiply by the percentage dry substance to obtain grams of total solids per 100 cc of sample. To obtain the percentage of total solids in the sample, divide the grams of total solids per 100 cc by the sp. gr. of the sample, 35.

38

## GLYCEROL

- (a) *Products containing 5 g per 100 cc or less of total solids*.—Proceed as directed under XV, 5 or 6.  
(b) *Products containing more than 5 g per 100 cc of total solids*.—Measure into a porcelain dish such a quantity of sample (not to exceed 100 cc) as contains 25 g or less of solid matter and evaporate on the steam bath to remove alcohol. Transfer to a 500 cc Erlenmeyer flask, using such a quantity of H<sub>2</sub>O that the final volume will be approximately 100 cc, and proceed as directed in XV, 7.

## SUCROSE

39

*Method I. By polarization*

Pipet into an evaporating dish the volume of sample equivalent to 52 g, as calculated from the sp. gr. as determined under 35; exactly neutralize with normal NaOH, calculating the quantity required from the determination of acidity, 41; evaporate on the steam bath to remove alcohol. Transfer to a 200 cc flask and proceed as directed under XXXIV, 22 or 23, beginning "add the necessary clarifying reagent, etc."

40

*Method II. By reducing sugars before and after inversion*

Approximate the sugar content of the sample from total solids, 37, and pipet into a porcelain dish such a quantity of sample as will contain 5–7 g of sugars; exactly neutralize with standard NaOH soln, calculating the quantity required from the acidity, and evaporate on the steam bath to remove alcohol. Transfer to a 200 cc volumetric flask, clarify with neutral Pb acetate soln, remove the excess Pb with K oxalate, and proceed as directed under XXXIV, 28, using the method given under XXXIV, 37, for the determination of reducing sugars.

41

## TOTAL ACIDITY

Place about 600 cc of H<sub>2</sub>O in an 800 cc beaker, add about 1 cc of phenolphthalein indicator, and titrate to a pink color with 0.1 N NaOH. Add 10–20 cc of sample

(unless this quantity gives the soln such a deep color that it will obscure the end point, in which case 5 cc may be used) and titrate to a pink color comparable to that of the soln before the sample was added. Calculate the acidity as g per 100 cc of sample in terms of the predominating acid present in the sample.

**42 PRELIMINARY PROCEDURE FOR CHARACTERISTIC ACIDS**

Measure out such a volume of sample as contains not more than 30 g of solid matter and not more than 200 mg of the acid to be determined, as calculated from the acidity; evaporate to about 30 cc, add 6 cc of 1 *N* NaOH, and let stand for at least 3 hours. Add 8 cc of 1 *N* H<sub>2</sub>SO<sub>4</sub>, transfer to a 250 cc volumetric flask, using 10 cc of H<sub>2</sub>O and sufficient 95% alcohol to fill the flask to the mark; mix and let stand 15 min. Filter thru a thin layer of absorbent cotton, protecting the liquid against evaporation. Transfer 200 cc of the filtrate to a centrifuge bottle and proceed with the determination of the acid as directed.

**43 TARTARIC ACID**

Using the material in the centrifuge bottle, proceed as directed under **XXVI, 27**.

**44 CITRIC ACID**

Using the material in the centrifuge bottle, proceed as directed under **XXVI, 31**.

**45 MALIC ACID**

Using the material in the centrifuge bottle, proceed as directed under **XXVI, 34**.

**46 VOLATILE ESTERS**

Measure 100–500 cc of sample into the distilling flask and proceed as directed under **XIII, 26**, collecting a volume of distillate at least twice as great as the volume of alcohol contained in the sample. (If determination **47** is to be made, use a 500 cc sample.) Disconnect the apparatus and wash out the condenser with a little H<sub>2</sub>O. Add about 1 cc of phenolphthalein indicator and titrate to a pink color which persists for at least 1 min., using 0.1 *N* NaOH or KOH. Add to the soln a measured excess of 25–50 cc of 0.1 *N* alkali, reflux for 1 hour, cool, and titrate the excess of alkali with 0.1 *N* H<sub>2</sub>SO<sub>4</sub>. Calculate the number of cc of 0.1 *N* alkali used in the saponification of the esters as ethyl acetate. 1 cc of 0.1 *N* = 8.8 mg of CH<sub>3</sub>COOC<sub>2</sub>H<sub>5</sub>.

**47 GAMMA UNDECALACTONE (QUALITATIVE)<sup>9</sup>**

(Peach and Apricot Cordials)

Make distinctly alkaline the soln obtained above under **46** and evaporate to dryness on the steam bath. Take up the residue in about 25 cc of H<sub>2</sub>O, transfer to a separatory funnel, acidify with H<sub>2</sub>SO<sub>4</sub> (1+1), let stand 10 min. to permit lactones to form, and extract 3 times with about 20 cc of ether. Unite the ether extracts and wash three times by shaking with 10 cc portions of normal Na<sub>2</sub>CO<sub>3</sub> soln. Permit the ether soln to evaporate spontaneously in a small beaker. To the residue add a few drops of N<sub>2</sub>H<sub>4</sub>·H<sub>2</sub>O soln (42% in H<sub>2</sub>O) and mix thoroly; if white solid matter separates out in a few minutes, gamma undecalactone is present. Allow the mixture to stand 15–20 min., place on the steam bath, and heat until the ammoniacal odor is no longer evident. Add 1 cc of normal butyl alcohol and warm until a clear soln is obtained, adding a few additional drops of the alcohol if necessary to dissolve the residue completely. Remove from the steam bath and permit the butyl alcohol to evaporate spontaneously. (This usually occurs overnight, but a longer time may be

necessary if much butyl alcohol has been used.) Examine the colorless or slightly yellowish crystals under the microscope. (Hydrazino- $\gamma$ -undecalactone has a characteristic odor similar to that of the lactone itself.)

#### 48 OPTICAL PROPERTIES OF HYDRAZINO- $\gamma$ -UNDECALACTONE

In ordinary light the substance is seen to consist of lath-like rods, many of them more or less split at the ends. In parallel polarized light (crossed nicols), the substance is characterized by not extinguishing sharply, most of the rods remaining essentially bright when the stage is rotated. Occasionally there are found crystals that extinguish sharply, have square ends, and show straight extinction and negative elongation. In convergent polarized light (crossed nicols) partial biaxial interference figures, usually showing one optic axis up or slightly inclined to the normal, are of frequent occurrence. The refractive indices, as determined by the immersion method, are as follows:  $\alpha = 1.483$  (not common);  $\beta = 1.525$  (most frequently occurring of the indices and shown lengthwise on rods);  $\gamma = 1.555$  (occurring crosswise on rods which show straight extinction and negative elongation); all  $\pm 0.003$ .

#### METHYL ALCOHOL

##### 49 PREPARATION OF SAMPLE

Measure into a distilling flask such a quantity of sample as contains 20-25 cc of absolute alcohol, add sufficient  $H_2O$  to make the total volume about 100 cc, and distil, collecting about 50 cc of distillate. To the distillate add 4 g of NaCl for each 10 cc of  $H_2O$ , and allow to stand several hours to reach the saturation point. Transfer to a separatory funnel, using about 10 cc of saturated NaCl soln to wash out the container, and shake with 25 cc of petroleum ether. When the separation is complete, transfer the  $H_2O$  soln to a second separatory funnel containing 25 cc of petroleum ether; shake, and transfer the  $H_2O$  soln to a third separatory funnel, also containing 25 cc of petroleum ether; shake and when the separation is complete, drain off the  $H_2O$  soln into a 200 cc distilling flask. In the meantime, add to the first funnel 25 cc of saturated NaCl soln and follow the sample thru with this soln, finally adding the washings to the sample soln in the distilling flask. Repeat this operation with a second 25 cc portion of saturated salt soln, finally adding this also to the distilling flask. Now distil the mixture into a 50 cc volumetric flask, using a suitable adapter. When 48-49 cc has distilled over, disconnect the apparatus and fill the flask to the mark with  $H_2O$ . Mix, and determine methyl alcohol as directed under 28, or under XXXIX, 103.

##### 50 ASH

Proceed as directed under XXVII, 8, using 25 cc of sample, the temp. of ashing not to exceed  $525^\circ$ .

##### 51 SOLUBLE AND INSOLUBLE ASH

Proceed as directed under XXXIV, 12.

##### 52 ALKALINITY OF SOLUBLE ASH

Proceed as directed under XXXIV, 13.

##### 53 ALKALINITY OF INSOLUBLE ASH

Proceed as directed under XXXIV, 14.

54

## PHOSPHORIC ACID

Evaporate 25 cc of sample to a sirupy consistency on the steam bath; add 7.5 cc of  $\text{Mg}(\text{NO}_3)_2$  soln, II, 7(e); mix thoroly, continue the evaporation as far as possible on the steam bath, and proceed as directed under XII, 28, beginning with, "heat on an electric hot plate at  $180^\circ$ ."

BENZALDEHYDE<sup>10</sup>

55

## REAGENT

*Phenylhydrazine soln.*—Add 1.5 cc of glacial acetic acid and 1 cc of newly distilled phenylhydrazine to 20 cc of  $\text{H}_2\text{O}$  and filter thru a moistened double white ribbon filter.

56

## DETERMINATION

Measure into a distilling flask such a quantity of sample as contains 30 cc of absolute alcohol, dilute to such a volume that the mixture will contain 300 cc of  $\text{H}_2\text{O}$  in addition to that required to dissolve the sugar present (1 g of sugar requires 0.5 cc of  $\text{H}_2\text{O}$ ), and distil off 300 cc into a 500 cc Erlenmeyer flask. Add 10 cc of the reagent and shake for 5 min. Filter on a Gooch with a thin mat, and wash with  $\text{H}_2\text{O}$  and finally with two 10 cc portions of 10% alcohol. Dry in a vacuum desiccator over  $\text{H}_2\text{SO}_4$  for 24 hours, excluding light, or at  $70^\circ$  under 100 mm or less of pressure for 2 hours. Wt. of precipitate  $\times 0.5408$  = benzaldehyde.

57

## CAMEL

Proceed as directed under 32.

58

## COAL TAR COLORS

Proceed as directed under XXI.

59

## ALDEHYDES

Measure 100–200 cc of sample into a distillation flask. If the solid content is 25 g per 100 cc or less, add 12.5–25 cc of  $\text{H}_2\text{O}$ ; if greater than 25 g per 100 cc, add 5 cc of  $\text{H}_2\text{O}$  for each 10 g of solid matter present, and distil slowly, collecting a volume of distillate equal to that of the sample, and proceed as directed under 11.

60

## FURFURAL

Treat a portion of the prepared distillate, 59, as directed under 13.

61

## FUSEL OIL

Treat 50 cc of the prepared distillate, 59, as directed under 17.

## SELECTED REFERENCES

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- <sup>2</sup> Abs. Analyst, **24**, 13, 211, 212 (1899).
- <sup>3</sup> Allen, Commercial Organic Analysis, 4th ed., **1**, 98 (1909).
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- <sup>7</sup> J. Assoc. Official Agr. Chem., **18**, 75 (1935); **19**, 82 (1936).
- <sup>8</sup> J. Ind. Eng. Chem., **18**, 304 (1926).
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- <sup>10</sup> Ibid., **19**, 75, 182 (1936).

## XVII. BAKING POWDERS AND BAKING CHEMICALS

### 1 PREPARATION OF SAMPLE—OFFICIAL

Remove the entire sample from the package, pass thru a 20-mesh sieve, and mix thoroly.

#### TOTAL CARBON DIOXIDE

##### *Gravimetric Method Using Knorr's Apparatus<sup>1</sup>—Official*

### 2 APPARATUS

Connect a flask by means of a ground-glass joint with a glass connection thru the upper part of which passes a dropping funnel, and join at the side with a Liebig condenser. Connect the mouth of the funnel by means of a perforated stopper with a soda lime tube. Connect the upper end of the condenser by a rubber joint with a train of absorption bulbs, the first one containing  $\text{H}_2\text{SO}_4$  for drying the gas passing into the next bulb, which contains a 33% KOH soln. Then connect to a third bulb containing  $\text{H}_2\text{SO}_4$  for the absorption of moisture escaping from the KOH bulb, then to a fourth bulb, also containing  $\text{H}_2\text{SO}_4$ , as a precaution to prevent moisture from the air being absorbed by the train. Connect the last bulb to an aspirator. Many analysts prefer to replace the last bulb by two U-tubes filled with sifted soda lime.

### 3 DETERMINATION

Place 0.5–2 g of the sample, the quantity depending upon the percentage of  $\text{CO}_2$  present, in the flask, which must be perfectly dry. Close the flask with the stopper which carries the funnel tube and the tube connecting with the absorption apparatus. Weigh separately the second and third absorption bulbs and attach them to the apparatus. If two soda lime tubes are used, weigh them separately and refill the first when the second increases materially in weight. Nearly fill the funnel tube with  $\text{H}_2\text{SO}_4$  (1+5) and place the soda lime tube in position. Aspirate air thru the absorption bulbs at a rate of about 2 bubbles per second. Open the stopper of the funnel and allow the acid to run slowly into the flask, taking care that the evolution of gas is so gradual as not materially to increase the current thru the bulbs. After all the acid has been introduced, close the stopcock, continue the aspiration, and gradually heat the contents of the flask to boiling. (While the flask is being heated the aspirator tube may be removed, altho when using ground-glass joints many analysts prefer to aspirate during the entire operation.) Continue the boiling for a few minutes after the  $\text{H}_2\text{O}$  has begun to condense; then remove the flame, open the stopcock, and continue aspiration while the apparatus cools. Remove the second and third bulbs and weigh. The increase in weight is due to  $\text{CO}_2$ .

##### *Gasometric Method<sup>2</sup> Using Chittick's Apparatus—Official*

### 4 REAGENTS

*Displacement soln.*—Dissolve 100 g of  $\text{NaCl}$  or  $\text{Na}_2\text{SO}_4 \cdot 10\text{H}_2\text{O}$  in 350 cc of  $\text{H}_2\text{O}$ . Add approximately 1 g of  $\text{NaHCO}_3$  and 2 cc of methyl orange indicator, VI, 3(f), and then sufficient  $\text{H}_2\text{SO}_4$  (1+5) to make just acid (a decided pink color). Stir until all  $\text{CO}_2$  is removed. This soln is used in the gas-measuring tube and leveling bulb and seldom needs to be replaced.

5

## APPARATUS

Connect a decomposition flask (*A*) by means of a glass T-tube (*B*), provided with a stopcock (*C*), to a graduated gas-measuring tube (*D*), which in turn is connected with a leveling bulb (*E*). For *A* always use a 250 cc wide-mouthed extraction flask of Pyrex or other resistant glass fitted with a two-holed rubber stopper, thru one hole of which passes the extended tip of a 25 cc buret (*F*) and thru the other a glass tube of the same diameter as the connecting T-tube. Use a buret graduated in cc at 20°, numbered at 5 cc intervals, and provided with an extra long tip bent to pass thru the rubber stopper. Connect the glass tube leading from the decomposition flask to the T-tube by means of rubber tubing to permit rotation of the flask. Use a gas-measuring tube graduated in cc at 20°, the zero mark being placed at a point 25 cc below the top marking to allow for graduating upwards from 0 to 25 cc and downward from 0 to 200 cc. By means of a long rubber tube connect the gas-measuring tube with the leveling bulb, which has a capacity of about 300 cc.

6

DETERMINATION<sup>3</sup>

Weigh 1.7 g of the prepared sample, 1, into flask *A* and connect this flask with the apparatus (Fig. 19). Open stopcock *C* and by means of the leveling bulb *E* bring the displacement soln to the 10 cc graduation above the zero mark. (This 10 cc is practically equal in volume to the volume of acid to be used in the decomposition.) Allow the apparatus to stand 1–2 min. to insure that the temp. and pressure within the apparatus are the same as those of the room. Close the stopcock, lower the leveling bulb somewhat to reduce the pressure within the apparatus, and slowly run into the decomposition flask from buret *F* 10 cc. of  $\text{H}_2\text{SO}_4$  (1+5). To prevent the liberated  $\text{CO}_2$  from escaping thru the acid buret into the air, keep the displacement soln in the leveling bulb at all times during the decomposition at a lower level than that in the gas-measuring tube. Rotate and then vigorously agitate the decomposition flask to secure intimate mixture of the contents. Allow to stand for 5 min. to secure equilibrium. Equalize the pressure in the measuring tube by means of the leveling bulb and read the volume of gas in the tube. Observe the temp. of the air surrounding the apparatus and also the barometric pressure at the time and multiply the number of cc of gas evolved by the factor

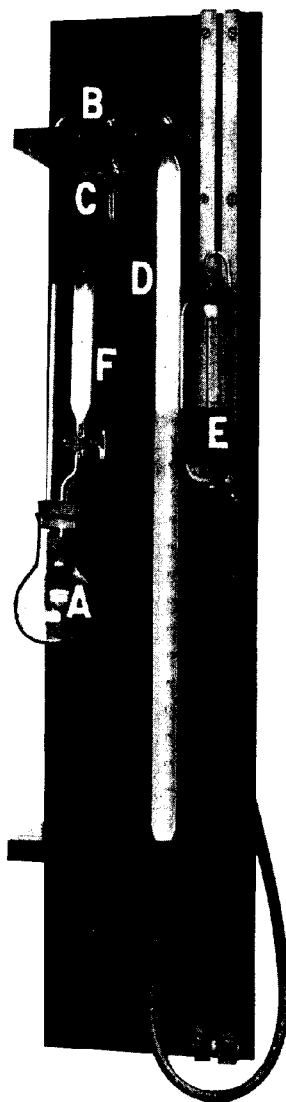


FIG. 19.—APPARATUS FOR THE GASOMETRIC DETERMINATION OF CARBON DIOXIDE

given in the table for this temp. and pressure, **XLII, 24**. Divide the corrected reading by 10 to obtain the percentage by weight of  $\text{CO}_2$  in the sample.

#### RESIDUAL CARBON DIOXIDE<sup>a</sup>

7

##### *Gravimetric Method—Official*

Weigh 2 g of the prepared sample, 1, into a flask suitable for the subsequent determination of  $\text{CO}_2$ ; add 20 cc of cold  $\text{H}_2\text{O}$ ; and allow to stand 20 min. Place the flask in a metal drying cell surrounded by boiling  $\text{H}_2\text{O}$  and heat, with occasional shaking, for 20 min. To complete the reaction and drive off the last traces of gas from the semi-solid mass, heat quickly to boiling and boil for 1 min. Aspirate until the air in the flask is thoroly changed, and determine the residual  $\text{CO}_2$  by absorption, as directed under 3.

8

##### *Gasometric Method<sup>a</sup>—Official*

Place 1.7 g of the prepared sample, 1, in the decomposition flask, A, Fig. 19; add 20 cc of  $\text{H}_2\text{O}$  and allow to stand 20 min. Place the flask in a metal drying cell surrounded by boiling  $\text{H}_2\text{O}$  and heat, with occasional shaking, for 20 min. To complete the reaction, heat quickly to boiling and boil for 1 min. Cool to room temp., connect the flask to the apparatus described under 5, and determine the  $\text{CO}_2$  present by treating with 10 cc of  $\text{H}_2\text{SO}_4$  (1+5) as directed under 6, using the correction factors given in Tables 25 and 26, **XLII**. To prevent foaming add 1-3 drops of caprylic alcohol to the baking powder in the decomposition flask.

9

#### AVAILABLE CARBON DIOXIDE—OFFICIAL

Subtract the residual  $\text{CO}_2$ , 7, from the total  $\text{CO}_2$ , 6.

#### NEUTRALIZING VALUE

10

##### *Of Acid-Reacting Materials Other Than Phosphates—Official*

Dissolve 1 g of the sample in hot  $\text{H}_2\text{O}$  and titrate with 0.2 N NaOH, using phenolphthalein indicator.

11

##### *Of Monocalcium Phosphate<sup>b</sup>—Tentative*

(Industrial method, results approximate.)

Weigh 0.84 g of monocalcium phosphate into a small beaker or casserole, add 25 cc of  $\text{H}_2\text{O}$  and 5 drops of a 0.2% soln of phenolphthalein,<sup>7</sup> and titrate with 0.2 N NaOH, to a faint pink. Heat to boiling, boil 1 min., and again continue the titration, while the soln is hot, to a faint pink color, adding the bulk of the standard alkali soln rapidly and with vigorous stirring. The total buret reading  $\times 2$  = the neutralizing strength of 100 parts of phosphate in terms of  $\text{NaHCO}_3$ .

12

#### TARTARIC ACID, FREE OR COMBINED (QUALITATIVE TEST)<sup>a</sup>—TENTATIVE

(Applicable in the presence of phosphates.)

Shake repeatedly about 5 g of the sample with about 250 cc of cold  $\text{H}_2\text{O}$  in a flask and allow the insoluble portion to subside. Decant the soln thru a filter and evaporate the filtrate to dryness. Powder the residue, add a few drops of 1% resorcinol soln, **XXXIV, 93**, and about 3 cc of  $\text{H}_2\text{SO}_4$ , and heat slowly. Tartaric acid is indicated by a rose-red color, which is discharged on dilution with  $\text{H}_2\text{O}$ .

13

#### TOTAL TARTARIC ACID—OFFICIAL

(Applicable only in the absence of Al salts, Ca salts, and phosphates.)

Into a 500 cc porcelain casserole or similarly shaped dish weigh 1.88 g of the prepared sample, 1. Add 10 cc of  $\text{H}_2\text{O}$  and then 10 cc of HCl (1+1) cautiously to avoid

loss due to the sudden evolution of  $\text{CO}_2$ . Heat gently until most of the starch present is hydrolyzed. Add slowly and with constant stirring 15 cc of  $\text{K}_2\text{CO}_3$  soln (328 g of  $\text{K}_2\text{CO}_3$  per liter), boil gently on a hot plate for 1 min., and evaporate on a steam bath to incipient crystallization. Remove from the steam bath and add, dropwise with constant stirring, 3 cc of glacial acetic acid. Add 2 cc more of the glacial acetic acid and continue the stirring for 3 min. Add 150 cc of 95% alcohol, carefully rinsing down the sides of the dish with the alcohol; stir vigorously for 5 min.; and let stand for at least 1 hour. Decant thru a Gooch crucible containing a thin layer of paper pulp or thru filter paper on a perforated disk, and wash, largely by decantation, with 95% alcohol until the combined filtrate and washings measure 550 cc. Test the last few cc of filtrate with dilute litmus tincture to be sure the precipitate has been properly washed. Return the paper pulp, or disk, containing a part of the precipitate to the residue in the porcelain dish and add 120 cc of hot  $\text{H}_2\text{O}$ . Add sufficient 0.2 *N* alkali soln to neutralize most, but not all, of the acidity. Boil the soln for 5 min. and complete the titration with 0.2 *N* alkali, using phenolphthalein indicator. The number of cc of 0.2 *N* alkali times 2 equals the percentage of K bitartrate to which 0.15% is to be added to compensate for loss due to solubility. 1 cc of 0.2 *N* alkali = 0.02641 g of tartaric anhydride, 0.03001 g of tartaric acid, and 0.03763 g of K bitartrate.

#### 14 FREE TARTARIC ACID (QUALITATIVE TEST)—OFFICIAL

Extract 5 g of the sample with absolute alcohol and evaporate the alcohol from the extract. Dissolve the residue in  $\text{NH}_4\text{OH}$  (1+10), transfer to a test tube, add a good sized crystal of  $\text{AgNO}_3$ , and heat gently. Tartaric acid is indicated by the formation of a silver mirror. If desired, the absolute alcoholic extract may be tested as directed under 12.

#### STARCH

15

##### *Direct Inversion Method—Official*

(For baking powders and baking chemicals free from calcium.)

Weigh 5 g of the sample into a 500 cc volumetric flask and proceed as directed under XXVII, 31.

16

##### *Indirect Method<sup>b</sup>—Official*

(For baking powders and baking chemicals containing calcium.)

Mix 5 g of the sample with 200 cc of  $\text{HCl}$  (1+11) in a 500 cc volumetric flask and allow the mixture to stand for an hour, shaking frequently. Filter on an 11 cm hardened filter, taking care to obtain a clear filtrate. Rinse the flask once without attempting to remove all the starch, and wash the paper twice with cold  $\text{H}_2\text{O}$ . Carefully wash the starch from the paper back into the flask with 200 cc of  $\text{H}_2\text{O}$ . Add 20 cc of  $\text{HCl}$  (sp. gr. 1.125) and proceed as directed under XXVII, 31. (The treatment with dilute hydrochloric acid (1+11), without dissolving the starch, removes effectively the Ca, which otherwise would be precipitated as tartrate by the alkaline Cu soln.)

17

##### *Modified McGill Method—Tentative*

Digest 1 g of the sample with 150 cc of  $\text{HCl}$  (1+11) for 24 hours at room temp., with occasional shaking. Filter on a Gooch crucible, wash thoroly with cold  $\text{H}_2\text{O}$  and then once with alcohol and once with ether. Dry at  $110^\circ$  (4 hours is usually sufficient), cool, and weigh. Burn off the starch, weigh again, and determine the



starch by difference. (The results by this method on cream of tartar powders and tartaric acid powders agree closely with those obtained by Cu reduction. The results on other types of baking powders are usually satisfactory, but in some instances they may be over 2% too high.)

#### ALUMINUM

##### *Qualitative Test<sup>10</sup>—Tentative*

(In presence of phosphates.)

#### 18

##### REAGENTS

- (a) *Hydrochloric acid soln.*—Approximately normal. Dilute 9 cc of HCl to 100 cc.
- (b) *Ammonium acetate soln.*—3 N. Dissolve 23.1 g of  $\text{NH}_4$  acetate in  $\text{H}_2\text{O}$  and dilute to 100 cc.
- (c) *Aurintricarboxylic acid soln.*—0.1%. Dissolve 0.1 g in  $\text{H}_2\text{O}$  and dilute to 100 cc.

#### 19

##### DETECTION

Dissolve 1–5 g of the baking powder in 5 cc of the N HCl and 5 cc of the 3 N  $\text{NH}_4$  acetate. Add 5 cc of the 0.1% soln of aurintricarboxylic acid, mix, and allow the lake formation to take place. Make the soln alkaline with  $\text{NH}_4\text{OH}$  containing a small quantity of  $(\text{NH}_4)_2\text{CO}_3$ . A bright persistent red precipitate indicates the presence of Al.

##### *By Precipitation with Phenylhydrazine<sup>11</sup>—Tentative*

#### 20

##### REAGENTS

- (a) *Ammonium bisulfite soln.*—Pass  $\text{SO}_2$  into a cool soln of  $\text{NH}_4\text{OH}$  (1+1) until the color of the soln becomes distinctly yellow.
- (b) *Phenylhydrazine bisulfite soln.*—To a few cc of phenylhydrazine add gradually a saturated soln of  $\text{SO}_2$  until the precipitate of phenylhydrazine sulfite, which at first separates out in crystals, is almost redissolved. If the precipitate is completely dissolved, add a drop or two of phenylhydrazine until a slight precipitate is obtained. Filter the soln before using. (From 5–10 cc of this soln in 100 cc of  $\text{H}_2\text{O}$  is sufficient strength for washing the  $\text{Al}(\text{OH})_3$  precipitate. If well stoppered, this concentrated soln of phenylhydrazine bisulfite will keep indefinitely.)

#### 21

##### DETERMINATION

Ignite 3 g of baking powder at a temp. not exceeding  $550^\circ$ . As soon as the C has burned off, take up the residue in HCl (4+10) and boil gently to assist soln. Filter into a 300 cc volumetric flask and wash with hot  $\text{H}_2\text{O}$ . Ignite the insoluble residue and filter paper in a Pt crucible and fuse the residue with about 2 g of  $\text{Na}_2\text{CO}_3$ . Dissolve the fused mass in  $\text{H}_2\text{O}$  and HCl and transfer to the volumetric flask containing the original filtrate. Cool, and make up to volume.

Transfer 100 cc aliquots to 400 cc beakers. Heat nearly to boiling, add  $\text{NH}_4\text{OH}$  (1+10) until a slight permanent precipitate forms, then just redissolve this precipitate with a drop or two of the dilute HCl. Add, dropwise with constant stirring, 10 or 12 drops of a saturated soln of  $\text{NH}_4\text{HSO}_3$ . Then add to the hot soln sufficient phenylhydrazine to precipitate the  $\text{Al}(\text{OH})_3$  completely (1 or 2 cc; an excess colors the soln yellow). If a permanent precipitate does not form at this point, add  $\text{NH}_4\text{OH}$  (1+10) carefully, dropwise, just to a permanent precipitate, and then complete the precipitation by adding a few more drops of the phenylhydrazine. Let stand a few min. for the precipitate to settle, then filter while still warm. Wash the pre-

precipitate with warm  $H_2O$  containing the phenylhydrazine bisulfite until the washings give no test for iron when yellow  $NH_4$  sulfide is added.

Place the filter paper containing the precipitate in a weighed Pt crucible. Dry, char, and ignite at a low temp. After the filter paper has completely burned, continue the ignition at a bright red heat to constant weight. Weigh quickly with the cover on the crucible as the precipitate is very hygroscopic. A second weighing is always necessary. The precipitate consists of  $Al_2O_3$  and Al phosphate.

Fuse the ignited precipitate with about 2 g of  $Na_2CO_3$  and dissolve the fusion in  $HNO_3$  (1+9). Transfer to a 250 cc beaker and boil to insure that all the phosphoric acid is in the ortho state. Cool. Transfer to a 200 cc flask, make up to volume, and use 50 cc aliquots to determine the  $P_2O_5$ . Multiply the weight of  $P_2O_5$  obtained by 4 and subtract the product from the weight of combined precipitates obtained above. The difference is the weight of  $Al_2O_3$  in 1 g of baking powder.

Weight  $Al_2O_3 \times 100 =$  percentage of  $Al_2O_3$ .

Percentage of  $Al_2O_3 \times 4.749 =$  percentage of  $Na_2Al_2(SO_4)_4$ .

If the baking powder contains a significant quantity of  $SiO_2$ , remove it by evaporating the HCl soln of the powder to dryness and dehydrating at  $105^\circ$  for 2 hours. Add to the dry mass 10 cc of HCl and 100 cc of  $H_2O$ , boil, filter off the  $SiO_2$ , and proceed as directed above.

## 22 INSOLUBLE ASH AND PREPARATION OF SOLUTION<sup>12</sup>—OFFICIAL

Char 5 g of the sample in a Pt dish at a heat below redness. Boil the carbonaceous mass with HCl (1+2.5), filter into a 500 cc volumetric flask, and wash with hot  $H_2O$ . Return the residue, together with the paper, to the Pt dish, and burn to a white ash. Boil again with the dilute HCl, filter, wash, unite the two filtrates, and dilute to 500 cc. Incinerate the residue after the last filtration and weigh the ash insoluble in acid.

## 23 IRON AND ALUMINUM<sup>12</sup>—OFFICIAL

Draw a 100 cc aliquot of the prepared soln, 22, and separate  $SiO_2$  if necessary. Mix the soln with 10% Na phosphate soln in excess. Add  $NH_4OH$  until a permanent precipitate is obtained, then HCl, dropwise, until the precipitate is dissolved. Bring the soln to a boil and boil for 2–3 min.; mix with a considerable excess of 50%  $NH_4$  acetate soln and 4 cc of 80% acetic acid. As soon as the precipitate of Al phosphate, mixed with Fe phosphate, has settled, collect on a filter, wash with hot  $H_2O$ , ignite, and weigh. Fuse the mixed phosphates with 10 parts of  $Na_2CO_3$ , dissolve in  $H_2SO_4$  (1+6), reduce with zinc, and determine the iron by titration with a standard permanganate soln (1 cc = 1 mg of Fe). In the same soln determine the phosphoric acid, as directed under II, 9 or 12. To obtain the weight of  $Al_2O_3$ , subtract the sum of the weights of  $Fe_2O_3$  and  $P_2O_5$  from the weight of the mixed phosphates.

## 24 CALCIUM<sup>12</sup>—OFFICIAL

Heat the combined filtrate and washings obtained under 23 to  $50^\circ$  and add an excess of saturated  $NH_4$  oxalate soln. Allow to stand in a warm place until the precipitate has settled, filter, wash the precipitate with hot  $H_2O$ , dry, and ignite over a Bunsen burner and finally over a blast lamp. Cool in a desiccator and weigh as  $CaO$ .

## 25 POTASSIUM AND SODIUM<sup>12</sup>—OFFICIAL

Evaporate an aliquot of the prepared soln, 22, nearly to dryness to remove the excess of HCl, dilute, and heat to boiling. While still boiling add 10%  $BaCl_2$  soln

as long as a precipitate forms and then enough saturated  $\text{Ba}(\text{OH})_2$  soln to make the liquid strongly alkaline. As soon as the precipitate has settled, filter and wash with hot  $\text{H}_2\text{O}$ ; heat the filtrate to boiling; add sufficient  $(\text{NH}_4)_2\text{CO}_3$  [1 part of  $(\text{NH}_4)_2\text{CO}_3$  in 5 of  $\text{NH}_4\text{OH}$  soln (1+12)] to precipitate all the Ba; filter, and wash with hot  $\text{H}_2\text{O}$ . Evaporate the filtrate to dryness and ignite the residue below redness to remove  $\text{NH}_4$  salts. Add to the residue a little  $\text{H}_2\text{O}$  and a few drops of the  $(\text{NH}_4)_2\text{CO}_3$  soln. Filter into a weighed Pt dish, evaporate, ignite below redness, and weigh the mixed K and Na chlorides. Determine K in the mixed chlorides as directed under II, 47, beginning with "Digest the residue with hot  $\text{H}_2\text{O}$ , filter thru a small filter." Calculate the K so found to its equivalent of KCl and subtract this result from the weight of the mixed chlorides to obtain the weight of NaCl.

## 26

## PHOSPHORIC ACID—OFFICIAL

Mix 5 g of the sample with a little  $\text{Mg}(\text{NO}_3)_2$  soln, II, 7(e), dry, ignite, dissolve in HCl (1+2.5), and dilute the soln to a definite volume. In an aliquot of the soln determine phosphoric acid as directed under II, 7 or 10.

## 27

SULFURIC ACID<sup>13</sup>—OFFICIAL

Boil 5 g of the sample for 1.5 hours with a mixture of 300 cc of  $\text{H}_2\text{O}$  and 15 cc of HCl. Filter, wash filter thoroly with hot  $\text{H}_2\text{O}$ , cool the combined filtrate and washings, and dilute to a volume of 500 cc. Determine  $\text{H}_2\text{SO}_4$  in an aliquot of 100 cc as directed under XII, 27.

## 28

## AMMONIA—OFFICIAL

Introduce 2 g of the sample into a distillation flask, add 300–400 cc of  $\text{H}_2\text{O}$  and an excess of NaOH soln (1+1), connect with a condenser, and distil into a measured volume of standard acid. Titrate the excess of acid in the distillate with standard alkali, using methyl red or cochineal indicator.

## 29

## ARSENIC—TENTATIVE

Introduce 5 g of the sample directly into the generator described under XXIX, 2; add 10 cc of  $\text{H}_2\text{O}$ , a little at a time to prevent foaming over, and then 15 cc of As-free HCl, introducing it dropwise until foaming ceases. Heat on a steam bath until a drop of the mixture, when diluted and treated with I soln, shows no blue color. Then dilute to about 30 cc with  $\text{H}_2\text{O}$  and continue from this point as directed under XXIX, 4, beginning with "Add 5 cc of the KI reagent." Make the blank and the standards for comparison by the use of the As-free HCl of the same concentration as that used in the determination.

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## **XVIII. COFFEE AND TEA**

### **GREEN COFFEE**

#### **1                   MACROSCOPIC EXAMINATION—TENTATIVE**

A macroscopic examination usually shows the presence of excessive quantities of black and blighted coffee beans, coffee hulls, stones, and other foreign matter. Separate these by hand picking and determine the quantity gravimetrically.

#### **2                   COLORING MATTERS—TENTATIVE**

Shake vigorously 100 g or more of the sample with cold H<sub>2</sub>O or alcohol, 70% by volume. Strain thru a coarse sieve and allow to settle. Identify soluble colors in the soln and insoluble pigments in the sediment as directed under **XXI**.

### **ROASTED COFFEE**

#### **3                   MACROSCOPIC EXAMINATION—TENTATIVE**

Artificial coffee beans are apparent from their regularity of form, and roasted legumes and lumps of chicory in whole roasted coffee can be picked out and identified microscopically. For ground coffee sprinkle some of the sample on cold H<sub>2</sub>O and stir lightly. Fragments of pure coffee, if not overroasted, will float, while fragments of chicory, legumes, cereals, etc., will sink immediately, chicory coloring the H<sub>2</sub>O a decided brown. In all cases identify the particles that sink by microscopical examination.

#### **4                   PREPARATION OF SAMPLE—OFFICIAL**

Grind the sample to pass thru a 30-mesh sieve and preserve in a tightly stoppered bottle.

#### **5                   MOISTURE—TENTATIVE**

Dry 5 g of the sample at the temp. of boiling H<sub>2</sub>O under a pressure not to exceed 100 mm of Hg, or at a temp. of 105–110° under atmospheric pressure, for 5 hours and subsequent periods of 1 hour each until constant weight is obtained. For whole coffee, grind rapidly to a coarse powder and without sifting and unnecessary exposure to the air weigh portions for the determination. For ground coffee, sample directly without further grinding.

#### **6                   SOLUBLE SOLIDS—TENTATIVE**

Place 4 g of the prepared sample, **4**, in a 200 cc flask. Add H<sub>2</sub>O to the mark, allow the mass to infuse for 8 hours, with occasional shaking, let stand 16 hours longer without shaking, filter, and evaporate 50 cc of the filtrate to dryness in a flat-bottomed dish. Dry at 100°, cool, and weigh.

#### **7                   ASH—OFFICIAL**

Proceed as directed under **XXVII**, **8**, using the sample prepared as directed under **4**.

#### **8                   SOLUBLE AND INSOLUBLE ASH—OFFICIAL**

Proceed as directed under **XXXIV**, **12**, using the ash obtained under **7**.

**9 ALKALINITY OF THE SOLUBLE ASH—OFFICIAL**

Proceed as directed under XXXIV, 13, using the filtrate obtained under 8.

**10 ASH INSOLUBLE IN ACID OFFICIAL**

Proceed as directed under XXXIII, 5, using the ash obtained as directed under 7 or the water-insoluble ash obtained as directed under 8.

**11 SOLUBLE PHOSPHORIC ACID IN THE ASH—OFFICIAL**

Proceed as directed under II, 9 or 12, using the soln obtained under 11.

**12 INSOLUBLE PHOSPHORIC ACID IN THE ASH—OFFICIAL**

Boil the insoluble ash as directed under 8 with 25 cc of HCl (1+2), filter, wash thoroly with hot H<sub>2</sub>O, and determine P<sub>2</sub>O<sub>5</sub> in combined filtrate and washings as directed under II, 9 or 12.

**13 CHLORIDES—OFFICIAL**

Proceed as directed under XII, 35.

**CAFFEINE****14 Power-Chesnut Method<sup>1</sup>—Official**

Moisten 10 g of the prepared sample, 4, with 95% alcohol; transfer to a Soxhlet or similar extraction apparatus; and extract with 95% alcohol for 8 hours, exercising care to assure complete extraction. Transfer the extract with the aid of hot H<sub>2</sub>O to a porcelain dish containing 10 g of heavy MgO in suspension in 100 cc of H<sub>2</sub>O. Evaporate slowly on a steam bath with frequent stirring to a dry, powdery mass. Rub the residue with a pestle into a paste with boiling H<sub>2</sub>O and transfer with hot H<sub>2</sub>O to a smooth filter, cleaning the dish with a rubber policeman. Collect the filtrate in a liter flask marked at 250 cc and wash with boiling H<sub>2</sub>O until the filtrate reaches the mark. Add 20 cc of H<sub>2</sub>SO<sub>4</sub> (1+9) and boil gently for 30 min. with a funnel in the neck of the flask. Cool, filter thru a moistened double paper into a separatory funnel, and wash with small portions of H<sub>2</sub>SO<sub>4</sub> (1+199). Extract with 6 successive 25 cc portions of CHCl<sub>3</sub>. Wash the combined CHCl<sub>3</sub> extracts in a separatory funnel with 5 cc of 1% KOH soln. Filter the CHCl<sub>3</sub> into an Erlenmeyer flask. Wash the KOH soln with 2 portions of CHCl<sub>3</sub> of 10 cc each, adding them to the flask, together with the CHCl<sub>3</sub> washings of the filter paper. Evaporate or distil on a steam bath to a small volume (10–15 cc), transfer with CHCl<sub>3</sub> to a weighed beaker, evaporate carefully, dry for 30 min. at 100°, and weigh. Test the purity of the residue by determining N and multiplying by the factor 3.464.

With products very low in caffeine combine the caffeine residues from duplicate determinations (representing 20 g of original material) and determine N as directed in II, 19 or 22, using half the quantity of reagents specified for the digestion and steaming out the apparatus thoroly before distilling. Distil to a small volume in the distilling flask to insure removal of all ammonia. Correct for the blank obtained, using the same reagents and apparatus, and pure sucrose in place of caffeine.

**15 Fendler-Stüber Method (Modified)<sup>2</sup>—Tentative**

(Adapted for quick results.)

Treat 10 g of the prepared sample, 4, with 10 cc of NH<sub>4</sub>OH (1+2) and 200 g of CHCl<sub>3</sub> in a glass-stoppered bottle; shake continuously by machine or hand for 30

min.; and chill in an ice bath. Pour the entire contents of the bottle on a 24 cm folded filter, covering immediately with a watch-glass. Collect the filtrate with the funnel resting directly in the neck of a flask (previously weighed with stopper) and having the flask surrounded with ice. Stopper as soon as the soln ceases to run from the funnel in a continuous stream and weigh. Evaporate on a steam bath, removing the last  $\text{CHCl}_3$  with a current of air. Digest the residue with 80 cc of hot  $\text{H}_2\text{O}$  for 10 min. on a steam bath, shaking frequently, and let cool. Treat the soln with 1%  $\text{KMnO}_4$  soln (20 cc for roasted and 10 cc for green) and let stand 15 min. at room temp., shaking occasionally. Add 2 cc of  $\text{H}_2\text{O}_2$  soln (100 cc of 3%  $\text{H}_2\text{O}_2$ , free of acetanilid, plus 1 cc of glacial acetic acid). If the liquid is still red or reddish, add the  $\text{H}_2\text{O}_2$  soln, 1 cc at a time, until the excess of  $\text{KMnO}_4$  is destroyed. Place the flask on a steam bath for 15 min. and add 0.5 cc portions of the  $\text{H}_2\text{O}_2$  soln until the liquid ceases to become lighter. Cool, and filter by suction thru a Gooch crucible, washing with cold  $\text{H}_2\text{O}$ . Transfer the filtrate to a separatory funnel and extract 6 times with 25 cc portions of  $\text{CHCl}_3$ . Evaporate the combined  $\text{CHCl}_3$  extracts to a small volume, transfer to a weighed beaker, finish evaporation, dry at  $100^\circ$  to constant weight (30 min. is usually sufficient), and weigh the residue as caffeine. The weight of the caffeine, multiplied by 2000 and divided by the weight of the  $\text{CHCl}_3$  aliquot obtained from the first filtration, equals the percentage of caffeine in the 10 g sample. Test the purity of the residue as directed in 14.

16

## CRUDE FIBER—OFFICIAL

Proceed as directed under XXVII, 27, using the sample prepared as directed under 4.

17

## STARCH—TENTATIVE

Extract 5 g of the prepared sample, 4, on a hardened filter with 5 successive 10 cc portions of ether; wash with small portions of 95% alcohol until a total of 200 cc has passed thru; and proceed as directed under XXVII, 33, beginning with the second sentence.

18

## SUGARS—TENTATIVE

Weigh 10 g of the prepared sample, 4, into a 250 cc volumetric flask; add 1 g of powdered  $\text{NH}_4\text{NaHPO}_4$ ; and proceed as directed under XXVII, 28-30. Determine the reduced Cu in the  $\text{Cu}_2\text{O}$  precipitate either volumetrically, as directed under XXXIV, 40, or electrolytically, as directed under XXXIV, 42.

19

## PETROLEUM ETHER EXTRACT—OFFICIAL

Dry 2 g of the prepared sample, 4, at  $100^\circ$ , extract with petroleum ether (b. p.  $35-50^\circ$ ) for 16 hours, evaporate the solvent, dry the residue at  $100^\circ$ , cool, and weigh.

20

## TOTAL ACIDITY—TENTATIVE

Treat 10 g of the prepared sample, 4, with 75 cc of alcohol, 80% by volume, in an Erlenmeyer flask; stopper; and allow to stand 16 hours, shaking occasionally. Filter, and transfer an aliquot of the filtrate (25 cc for green coffee, 10 cc for roasted coffee) to a beaker; dilute to about 100 cc with  $\text{H}_2\text{O}$ ; and titrate with 0.1 N alkali, using phenolphthalein indicator. Express the result as the number of cc of 0.1 N alkali required to neutralize the acidity of 100 g of the sample.

21

## VOLATILE ACIDITY—TENTATIVE

Into a volatile acid apparatus, XV, 24, introduce a few glass beads and over these place 20 g of the unground sample. Add 100 cc of recently boiled  $\text{H}_2\text{O}$ , place

a sufficient quantity of recently boiled  $H_2O$  in the outer flask, and distil until the distillate is no longer acid to litmus paper (usually 100 cc of distillate will be collected). Titrate the distillate with 0.1 *N* alkali, using phenolphthalein indicator. Express the result as the number of cc of 0.1 *N* alkali required to neutralize the acidity of 100 g of the sample.

## COATING AND GLAZING SUBSTANCES

### 22

#### SUGAR AND DEXTRIN—TENTATIVE

Introduce 100 g of the whole coffee into a beaker, add exactly 300 cc of  $H_2O$ , stir, and allow to stand 5 min., stirring frequently. Filter thru a dry filter and add carefully to the filtrate dry Pb acetate until precipitation is complete, avoiding an excess of the reagent. Filter thru a dry filter and remove the Pb from the filtrate by the addition of a slight excess of dry, powdered K oxalate. Filter thru a dry filter and determine reducing sugars as invert sugar in 50 cc of the filtrate, as directed under XXXIV, 37. Invert a 75 cc aliquot of the filtrate as directed under XXXIV, 23(b). Cool, nearly neutralize with NaOH soln (1+1), dilute to 100 cc, and determine reducing sugars as invert sugar in the resulting soln as directed under XXXIV, 37. Measure a 100 cc aliquot of the filtrate into a 200 cc flask, add 10 cc of HCl (sp. gr. 1.125), and hydrolyze as directed under XXVII, 31. Cool, neutralize with NaOH soln (1+1), dilute to volume, filter thru a dry filter, and determine reducing sugars as invert sugar in 50 cc of the filtrate as directed under XXXIV, 37. Calculate the reducing sugars in each instance to percentage by weight of the original coffee: Calculate sucrose from the reducing sugars before and after inversion as directed under XXXIV, 28, and calculate dextrin as follows: Subtract the reducing sugars after inversion from the reducing sugars after hydrolysis and multiply the difference by the factor 0.8605 to convert the result to dextrin.

In some instances the presence of sucrose in the water extract may be verified by polarization. The presence of dextrin in the water extract may be verified by polarization as directed under XXXIV, 30, and by the erythro dextrin test (XXXIV, 91) made on the water extract previous to clarification with Pb acetate.

### 23

#### EGG ALBUMIN AND GELATIN—TENTATIVE

Treat 100 g of the whole coffee with 500 cc of  $H_2O$  and allow to stand for 5 min., stirring frequently. Filter, and treat separate portions of the filtrate with (1) a 5% soln of tannic acid, and (2) Millon's reagent (XX, 18). Boil a third portion of the filtrate. In the presence of egg albumin a more or less heavy precipitate will be formed in each case. As a confirmatory test, treat an aliquot of the filtrate with an excess of tannic acid soln; add a little salt if necessary to secure flocculation of the precipitate; filter; and, without washing, introduce the paper and its contents into a Kjeldahl flask and determine N. By this method coffee not coated with albumin or gelatin will yield less than 10 mg of N per 100 g of sample.

### 24

#### CHICORY INFUSION—TENTATIVE

Cover 100–150 g of the whole coffee with  $H_2O$ ; allow to soak 2–3 min., stirring frequently; and drain the aqueous washings thru a coarse sieve. Wash the coffee upon the sieve with about 100 cc of  $H_2O$  and centrifuge the combined washings. Decant the clear liquid from the sediment, which should then be drained almost dry on filter paper. Mount the sediment in chloral hydrate soln, XXXIII, 28(c), and examine under the microscope for elements of chicory.

25

## FATS AND WAXES—TENTATIVE

Treat 100–200 g of the beans with low-boiling petroleum ether for 10 min., pour off the petroleum ether, and repeat the process. Filter the combined extracts, evaporate, and determine the index of refraction and the saponification number of the residue as directed under XXXI, 9 and 23.

## TEA

26

## DUST, STEMS, AND FOREIGN LEAVES—TENTATIVE

Place 1 g of the tea in a 300 cc casserole, add 200 cc of boiling  $H_2O$ , and allow to stand 15 min. This treatment will cause the leaves to unroll, and they will then be in condition for examination as to form and structure.<sup>5</sup> A macroscopic examination will reveal the presence or absence of dust or stems. Only those stems that remain floating after the leaf is thoroly infused should be regarded as woody stems<sup>6</sup> ("floaters").

27

## PREPARATION OF SAMPLE—OFFICIAL

Grind the sample to pass thru a 30-mesh sieve.

28

## MOISTURE—OFFICIAL

Proceed as directed under XXVII, 2.

29

## WATER EXTRACT—OFFICIAL

To 2 g of the ground sample in a 500 cc volumetric flask, add 200 cc of hot  $H_2O$  and boil over a low flame for 1 hour, rotating occasionally. Close the flask with a rubber stopper thru which passes a glass tube 30 in. long for a condenser. Boil very slowly so that no steam escapes from the top of the air condenser. Cool, dilute to volume, mix thoroly, and filter thru a dry filter paper. Transfer an aliquot of 50 cc to a weighed dish and evaporate to dryness on a steam bath. Place in the oven, heat at 100° for 1 hour, cool, and weigh.

30

## ASH—OFFICIAL

Proceed as directed under XXVII, 8.

31

## SOLUBLE AND INSOLUBLE ASH—OFFICIAL

Proceed as directed under XXXIV, 12, using the ash obtained under 30.

32

## ALKALINITY OF THE SOLUBLE ASH—OFFICIAL

Proceed as directed under XXXIV, 13, using the filtrate obtained under 31.

33

## ALKALINITY OF THE INSOLUBLE ASH—OFFICIAL

Proceed as directed under XXXIV, 14, using the insoluble ash obtained under 31.

34

## ASH INSOLUBLE IN ACID—OFFICIAL

Proceed as directed under XXXIII, 5, using the total ash obtained as directed under 30, or the insoluble residue obtained under 31.

35

## SOLUBLE PHOSPHORIC ACID IN THE ASH—OFFICIAL

Proceed as directed under II, 9 or 11, using the soln of soluble ash obtained under 32.

36

## INSOLUBLE PHOSPHORIC ACID IN THE ASH—OFFICIAL

Proceed as directed under II, 9 or 11, using the soln obtained under 33.



37

## PETROLEUM ETHER EXTRACT—OFFICIAL

Proceed as directed under 19.

38

## PROTEIN—TENTATIVE

Determine N as directed under II, 21, 23 or 25. To obtain the percentage of N present as protein, subtract the percentage of N present as caffeine from the percentage of total N. Multiply this result by 6.25 to obtain the percentage of protein.

39

## CRUDE FIBER—OFFICIAL

Proceed as directed under XXVII, 27.

40

## VOLATILE OIL—TENTATIVE

Add 100 g of tea to 800 cc of  $H_2O$ , distil, extract the distillate several times with petroleum ether, transfer the combined petroleum ether extracts to a weighed dish, evaporate at room temp., dry in a desiccator, and weigh.

## CAFFEINE

41

*Power-Chesnut Method*<sup>3</sup>—Official

Proceed as directed under 14.

42

*Bailey-Andrew Method*<sup>3</sup>—Official

To 5 g of the prepared sample, 27, in a 500 cc volumetric flask, add 10 g of heavy  $MgO$  and 200 cc of  $H_2O$ . Boil gently over a low flame for 2 hours, using a small-bore glass tube 30 in. long as a condenser. Cool, dilute to volume, and filter thru a dry paper. Transfer an aliquot portion of 300 cc, equivalent to 3 g of original material, to an Erlenmeyer flask of 1 liter capacity; add 10 cc of  $H_2SO_4$  (1+9); and boil until the volume is reduced to about 100 cc. Filter into a separatory funnel, washing the flask with small portions of  $H_2SO_4$  (1+99), and shake 6 times with  $CHCl_3$ , using 25, 20, 15, 10, 10, 10 cc portions. Treat the combined extracts with 5 cc of a 1% soln of  $KOH$  and when the liquids have completely separated draw off the  $CHCl_3$  layer into a suitable flask or beaker. Wash the alkaline soln in the separatory funnel with 2 portions of  $CHCl_3$ , of 10 cc each, and unite the washings with the main bulk of extract. Evaporate or distil off the  $CHCl_3$  to a small bulk, transfer to a weighed flask, evaporate to dryness, and further dry in an oven at  $100^\circ$  to constant weight. Test the purity of the residue by determining N and multiplying by the factor 3.464. This gives a value for anhydrous caffeine.

TANNIN<sup>a</sup>—TENTATIVE

43

## REAGENTS

(a) *Potassium permanganate soln.*—Prepare a soln containing 1.33 g per liter and obtain its equivalent in terms of 0.1 N oxalic acid.

(b) *Indigo carmine soln.*—Prepare a soln containing 6 g of indigo carmine (free from indigo blue) and 50 cc of  $H_2SO_4$  per liter.

(c) *Gelatin soln.*—Soak 25 g of gelatin for 1 hour in saturated  $NaCl$  soln, heat until the gelatin is dissolved, cool, and dilute with a saturated  $NaCl$  soln to 1 liter.

(d) *Acid sodium chloride soln.*—Acidify 975 cc of saturated  $NaCl$  soln with 25 cc of  $H_2SO_4$ .

44

## DETERMINATION

Boil 5 g of the tea for 30 min. with 400 cc of  $H_2O$ , cool, transfer to a 500 cc volumetric flask, and dilute to the mark. To 10 cc of the infusion (filtered, if not clear), add 25 cc of the indigo carmine soln and about 750 cc of  $H_2O$ . Add the  $KMnO_4$  soln from a buret, a little at a time while stirring, until the color becomes light green, then dropwise until the color changes to bright yellow or to a faint pink at the rim. Designate the number of cc of  $KMnO_4$  used as "a."

Mix 100 cc of the clear infusion of tea with 50 cc of the gelatin soln, 100 cc of the acid NaCl soln, and 10 g of powdered kaolin, and shake several min. in a stoppered flask. After allowing the mixture to settle, decant thru a filter. Mix 25 cc of the filtrate with 25 cc of the indigo carmine soln and about 750 cc of H<sub>2</sub>O and titrate with KMnO<sub>4</sub> as before. The number of cc of KMnO<sub>4</sub> used subtracted from that obtained above, "a," gives the quantity of KMnO<sub>4</sub> required to oxidize the tannin. 1 cc of 0.1 N oxalic acid = approximately 0.0042 g of tannin (gallotannic acid).

## FACING

45

## GENERAL—TENTATIVE

(1) Examine the ash obtained as directed under 30 for mineral pigments (cf. **XXI**, 1); (2) shake a quantity of the tea with a large volume of H<sub>2</sub>O and remove the leaves by means of a sieve. Allow the insoluble matter in the H<sub>2</sub>O portion to settle, filter, and examine the residue on the filter paper for insoluble pigments as directed under **XXI**, 1. Catechu and other soluble substances, if used, will be found in the filtrate.

46

## PARAFFIN AND WAXY SUBSTANCES—TENTATIVE

Spread a quantity of the tea between two sheets of unglazed white paper and place thereon a hot iron. Any greasy substance will stain the paper.<sup>11</sup>

47

## PIGMENTS USED FOR COLORING OR FACING—TENTATIVE

Place 60 g of the tea in a 60-mesh, 5-6 inch sieve provided with a top. Sift a small quantity (approximately 0.1 g) of the dust upon a piece of semi-glazed, white paper about 8 by 10 inches. (To obtain the requisite quantity of dust, it is sometimes necessary to rub the leaf gently against the bottom of the sieve.) Place the paper on a plain, firm surface, preferably glass or marble, and crush the dust by pressing firmly upon it a flat steel spatula about 5 inches long. Repeat the crushing process until the tea dust is ground almost to a powder, when particles of coloring matter, if present, become visible as streaks on the paper. Brush off the loose dust and examine the paper by means of a simple lens magnifying 7.5 diameters. Bright light is essential to distinguish these particles and streaks. In many cases the character of the pigment is indicated by the behavior of these streaks when treated with reagents and examined under a microscope. The crushed particles of leaf of either black or green tea appear in such quantity that there is no chance of mistaking them for coloring or facing material. Repeat this test, using black, semi-glazed paper for facings such as talc, gypsum, BaSO<sub>4</sub>, or clay.

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## XIX. CACAO BEAN AND ITS PRODUCTS

### 1 PREPARATION OF SAMPLE—OFFICIAL

Mix powdered products thoroly and preserve in tightly stoppered bottles. Chill sweet or bitter chocolate until it becomes hard and reduce to a finely granular condition by grating or shaving. Mix thoroly and preserve in a tightly stoppered bottle in a cool place.

### 2 MOISTURE—TENTATIVE

Dry 2 g of the prepared sample, 1, to constant weight in a Pt dish in an air oven at 100°. (An Al dish may be used when the ash is not determined on the same sample.) Report the loss in weight as moisture.

### 3 ASH—OFFICIAL

Proceed as directed under XXVII, 8, using sufficient sample to contain approximately 1 g of water-, sugar-, and fat-free material.

### 4 SOLUBLE AND INSOLUBLE ASH—OFFICIAL

Proceed as directed under XXXIV, 12, using the ash obtained under 3.

### 5 ALKALINITY OF THE SOLUBLE ASH—OFFICIAL

Proceed as directed under XXXIV, 13, using the filtrate from 4.

### 6 ALKALINITY OF THE INSOLUBLE ASH—OFFICIAL

Proceed as directed under XXXIV, 14, using the insoluble ash obtained under 4.

### 7 ASH INSOLUBLE IN ACID—OFFICIAL

Proceed as directed under XXXIII, 5, using the total ash as obtained under 3, or the water-insoluble residue as obtained under 4.

### 8 TOTAL NITROGEN—OFFICIAL

Proceed as directed under II, 21, 23 or 25.

### 9 MILK PROTEINS—TENTATIVE

Weigh exactly 10 g of the finely grated chocolate into a suitable 8 oz centrifuge bottle. Add two 100 cc portions of ether, centrifuging and decanting the supernatant liquor after each addition. Dry the residue in an oven at about 100° and powder the residue in the bottle with a flattened glass rod. Add 200 cc of 3%  $\text{Na}_2\text{C}_2\text{O}_4$  and let stand 4 hours, shaking frequently. Centrifuge and filter thru a small folded filter. Discard the first 5–10 cc of the filtrate and determine N in 50 cc of this filtrate. Pipet 100 cc of the filtrate into a 200 cc volumetric flask and dilute almost to the mark with  $\text{H}_2\text{O}$ . Precipitate the proteins by the addition of 2 cc of glacial acetic acid. Make to volume, shake, filter, and determine N in 100 cc of the filtrate. The difference between the two N figures obtained is the N of the casein contained in 2.5 g of the sample. This figure  $\times 4 \times 6.38$  = the total casein contained in the 10 g taken for the analysis. Casein  $\times 1.25$  = total milk protein.

### 10 SUCROSE

Transfer 26 g of the prepared sample, 1, to an 8 oz nursing bottle, add about 100 cc of petroleum ether, shake 5 min., and centrifuge. Decant the clear solvent care-

fully and repeat the treatment with petroleum ether. Place the bottle containing the defatted residue in a warm place until the petroleum ether is expelled. Add 100 cc of  $H_2O$  and shake until most of the chocolate is detached from the sides and bottom of the bottle. Loosen the stopper and carefully immerse the bottle for 15 min. in a water bath kept at  $85-90^\circ$ , shaking occasionally to remove all the chocolate from the sides of the bottle. Remove from the water bath, cool, and add basic Pb acetate soln (sp. gr. 1.25) to complete precipitation (5 cc is usually sufficient). Add  $H_2O$  to make a total volume of 110 cc of added liquid. Mix thoroly, centrifuge, and decant the supernatant liquid thru a small filter. Precipitate the excess of lead with powdered dry K oxalate and filter. Dilute sufficient filtrate with an equal volume of  $H_2O$ , mix, and polarize in a 200 mm tube at  $20^\circ$ . Obtain the invert reading at  $20^\circ$  as directed under XXXIV, 23(b). Multiply both readings by 2 to correct for dilution. From the data obtained calculate the percentage of sucrose (S) from the following formulas:

$$S = \frac{(P-I)(110+X)}{143.0-t/2}, \text{ in which}$$

$$X = \frac{0.2244(P-21d)}{1-0.00204(P-21d)}, \text{ in which}$$

$$d = \frac{P-I}{143.0-t/2}.$$

## 11

## LACTOSE IN MILK CHOCOLATE

Determine reducing sugars before inversion as directed under XXXIV, 37, in an aliquot (usually 20 cc) of the Pb-free filtrate obtained in 10. Determine reduced Cu as cuprous oxide by the volumetric thiosulfate method as directed under XXXIV, 40. Correct for the cuprous oxide due to the sucrose as follows:

Obtain the approximate percentage of lactose from the following formula, using the data obtained in 10.

$$\text{Approximate lactose} = \frac{P(1.1 + X/100) - S}{0.79}.$$

From the calculated polarimetric sucrose/lactose ratio and the total cuprous oxide obtained as above, determine the amount of cuprous oxide to be subtracted from the total cuprous oxide found, using the plot (Fig. 20). Convert the corrected cuprous oxide to lactose (L), using Table 9, XLII. The percentage of lactose is then obtained from the following relationship:

$$\text{Percentage lactose} = \frac{L(110+X)}{0.26 C}$$

in which X is the value obtained in the polarimetric sucrose determination and C is the volume of soln (cc) used in the above lactose determination.

## FAT

## 12

Method I.<sup>4</sup>—Official

Prepare in a Knorr extraction tube a tightly packed mat of asbestos purified as for the determination of crude fiber, XXVII, 25(c), and carefully freed from coarse pieces. Wash the filter with alcohol, ether, and a little petroleum ether. (All petroleum ether used in this determination must be redistilled below  $60^\circ$ .) Weigh 2-3

g of the prepared sample, 1, into the tube and insert the tube into a rubber stopper in a filtering bell-jar connected to the suction thru a two-way stopcock, taking care that no rubber particles adhere to the tip of the stem. Place a weighed 150 cc Erlenmeyer flask at such a height that the tube stem passes thru the neck into the flask. (The stem of the tube should be lengthened if necessary.) Fill the tube to about  $\frac{2}{3}$  of its capacity with the redistilled petroleum ether, and by means of a rod having a flattened end stir the sample thoroly, taking care to crush all lumps. Let stand

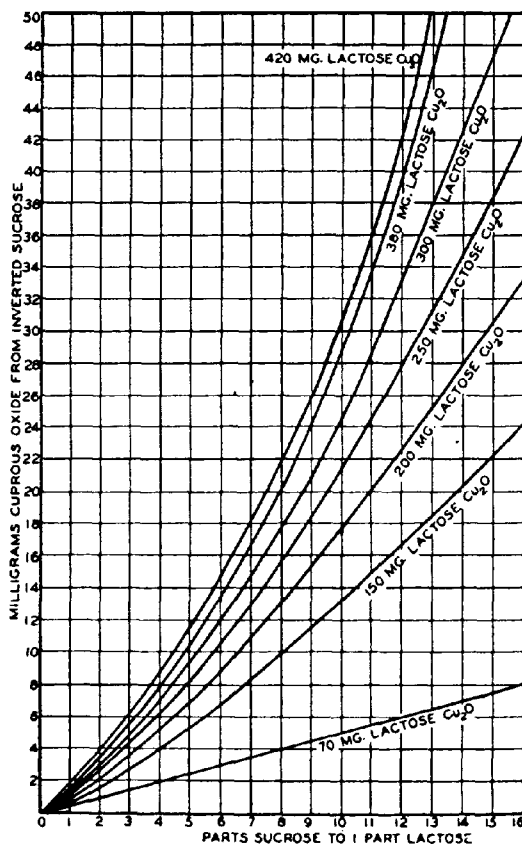


FIG. 20.—GRAPH USED IN CORRECTING CUPROUS OXIDE FOR EFFECT OF SUCROSE

1 min. and drain by suction. Regulate the suction so that the collected solvent in the flask will not boil violently. Add the solvent from a wash-bottle, at the same time turning the tube between thumb and finger so that the sides of the tube are washed down by each addition. Repeat the extractions, with stirring, until the fat is removed (10 extractions will usually be sufficient). Remove the tube with stopper from the bell, wash the traces of fat from the end of the stem with petroleum ether, evaporate the solvent, and dry to constant weight at 100°.

The fat-free sample may be used for the crude fiber determination.

13

*Method II.<sup>5</sup>—Tentative*

Weigh accurately about 2 g of the prepared sample, 1, and without previous drying stratify the charge in an extraction tube with about 0.5 g of asbestos, **XXVII**, **25(c)**, further washed with alcohol, ether, and petroleum ether. Extract with petroleum ether, redistilled below 60°, in a continuous extractor for 4 hours. Grind the material, to break up any lumps that may have formed, and re-extract for at least 4 hours. (It is advisable to allow the solvent to run thru the material once completely before applying heat for the continuous extraction.) Collect the petroleum ether extract in a weighed flask, evaporate the solvent, and dry the residue to constant weight at 100°. The extracted residue in the extraction tube may be used for the determination of crude fiber.

14

**MILK FAT IN MILK CHOCOLATE—TENTATIVE**

Estimate the quantity of milk fat in milk chocolate from the following formula based on a Reichert-Meissl number of 0.5 for cacao butter:

$$C = \frac{24A + 0.5B}{5}, \text{ in which}$$

$A$  = g of butter fat in 5 g of mixed fat;

$B = 5 - A$  = g of cacao fat in 5 g of mixed fat; and

$C$  = Reichert-Meissl number of extracted fat.

From which the

$$\text{Weight of butter fat in 5 g of mixed fat} = \frac{C - 0.5}{4.7}, \text{ and the}$$

$$\text{Percentage of butter fat} = \text{percentage of total fat} \times \frac{C - 0.5}{23.5}.$$

15

**SEPARATION AND PREPARATION OF FAT—TENTATIVE**

Separate the fat from 10–40 g of sample (depending upon the fat content) by shaking the material with 2 or 3 100 cc portions of ethyl ether. Centrifuge and decant each portion. Combine the portions in a beaker and drive off most of the ether on a steam bath. Filter the ether extracts thru a dry, folded filter and dry at 100°.

**DETECTION OF COCONUT AND PALM KERNEL OILS IN CACAO BUTTER AND FAT EXTRACTED FROM MILK CHOCOLATE<sup>6</sup>—TENTATIVE**

16

(a) *Examination of cacao butter.*—Saponify 5 g of the sample with 15 cc of alcoholic KOH soln (25 g to 200 cc of alcohol) and evaporate the alcohol on a steam bath. Run a blank on pure cacao butter at the same time. Add 5 cc of H<sub>2</sub>O and again evaporate to remove the last trace of alcohol. Dissolve the soap in 100 cc of H<sub>2</sub>O, cool to room temp., and add, while stirring, 100 cc of saturated salt soln. Allow to stand for 15 min., stirring several times during this period, and then separate the soap by filtration, using a Büchner funnel. To 100 cc of the filtrate add, while stirring, 100 cc of the saturated salt soln and allow to stand for 15 min. (Only a slight precipitate should appear.) Filter, add to the filtrate a drop of phenolphthalein indicator, neutralize with HCl (1 + 3), and add 0.5 cc of this reagent in excess. If the sample consists of pure cacao butter, the soln when acidified will remain clear; if coconut or palm kernel oil is present, the soln will become turbid or milky.

(b) *Examination of fat extracted from milk chocolate.*<sup>7</sup>—Milk fat, if present in cacao butter subjected to this test, produces a turbidity less in intensity than that produced by the same percentage of coconut or palm kernel oil. For example, cacao butter containing 10, 15, or 20% of milk fat produces, respectively, no opalescence, a faint opalescence, or an opalescence. For this reason, when the fat to be examined has been extracted from a cacao product that contains lactose or casein, multiply the percentage of lactose in the cacao product by 0.8, or the percentage of casein by 1.1, to obtain the percentage of milk fat in the product, and from this result calculate the percentage of milk fat in the total fat. If this percentage corresponds to 15% or less, a blank of cacao butter containing 15% milk fat may be used; otherwise make up a mixture of cacao butter and milk fat in the proportions indicated by the calculations.

Test the fat extracted from the sample under examination as directed under 16(a), but use the prepared mixture of cacao butter and milk fat instead of the pure cacao butter for the blank. If the fat being tested contains coconut oil or palm kernel oil, the last filtrate, when acidified, will be more turbid or milky than the blank.

#### CRITICAL TEMPERATURE OF DISSOLUTION OF FAT IN ACETIC ACID TEST<sup>8</sup>—TENTATIVE

##### 17

##### APPARATUS

Insert a thermometer reading to 0.1° into a cork that fits a 6× $\frac{1}{4}$  inch test tube and extend it far enough into the tube so that the bulb will be covered by 10 cc of liquid. Place the test tube in a larger tube (4×1 $\frac{1}{4}$  inch) containing glycerol and hold firmly in place with a cork having a groove cut in the side to equalize the pressure when heat is applied.

##### 18

##### DETERMINATION

To remove traces of moisture, filter a portion of the sample to be examined thru a dry paper in an oven in which a temp. of about 110° is maintained. Allow the filtered sample to cool until barely warm and weigh 5 g of the sample and 5 g of 99.5% acetic acid into the test tube. Insert the cork holding the thermometer and place the test tube in the glycerol bath. Heat, and shake the apparatus frequently until a clear soln of the fat and acetic acid is obtained. Allow the soln to cool, with constant shaking, without removing from the glycerol bath. Note the temp. at which the first sign of turbidity appears. Make a similar test with the same acetic acid on a sample of pure cacao butter.

Free fatty acids lower the turbidity temp. A correction, therefore, must be made for the acid value of the sample. If the strength of the acetic acid reagent is such that the turbidity temp. of the pure cacao butter is approximately 90°, one unit of acid value will cause a reduction of 1.4° in the critical temp. of dissolution. If the turbidity temp. is approximately 100°, one unit of acid value will cause a reduction of 1.2°. For intermediate temp. the reduction is proportional.

Determine the acid value (mg of KOH required to neutralize the free fatty acids in 1 g of the sample) of both the sample and the pure cacao butter as directed under XXXI, 30, using 5 g of fat. Multiply the acid value by the correction factor and add the result to the observed turbidity temp. The figure obtained is the true critical temp. of dissolution. If the true critical temp. of dissolution of the sample is lower than that of the pure cacao butter by more than 3° in the case of fat from chocolate liquors or sweet chocolates, and by more than 6° in the case of fat from milk chocolates, adulteration with coconut, palm kernel, corn, peanut, cottonseed oils, etc., or their stearines, is indicated.

**19 ACETONE-CARBON TETRACHLORIDE TEST OF FAT<sup>s</sup>—TENTATIVE**

Dissolve 5 cc of the warm fat, which has been previously filtered thru a dry filter paper in an oven at about 110° to remove traces of moisture, in 5 cc of acetone-CCl<sub>4</sub> reagent (equal quantities of each) in a test tube. Allow the soln to stand in ice H<sub>2</sub>O for 20–30 min. Run a blank on a sample of pure cacao butter at the same time. If hydrogenated oil, tallow, oleostearin, or paraffin is present, a white flocculent precipitate will soon appear. If the H<sub>2</sub>O is cold enough, cacao butter may solidify. If a precipitate is formed, remove the sample from the ice H<sub>2</sub>O and allow to remain at room temp. for a time. Solidified cacao butter will soon melt and go into soln, but if the precipitate is due to any of the above-mentioned possible adulterants a much longer time will be required.

**20 MELTING POINT—OFFICIAL**

Proceed as directed under **XXXI, 14**. Keep the fat at least 24 hours in a cool place before making the determination.

**21 INDEX OF REFRACTION—OFFICIAL**

Proceed as directed under **XXXI, 9**.

**22 IODINE ABSORPTION NUMBER—OFFICIAL**

Proceed as directed under **XXXI, 19** or **21**.

**23 SAPONIFICATION NUMBER—OFFICIAL**

Proceed as directed under **XXXI, 23**.

**24 REICHERT-MEISSL AND POLENSKE VALUES<sup>s</sup>—OFFICIAL**

Proceed as directed under **XXXI, 27**.

**SILVER NUMBER FOR DETECTION OF COCONUT AND PALM KERNEL OILS<sup>ss</sup>—TENTATIVE****25 REAGENTS**

- (a) *Potassium hydroxide soln.*—750 g of KOH per liter.
- (b) *Magnesium sulfate soln.*—150 g of MgSO<sub>4</sub>·7H<sub>2</sub>O per liter.
- (c) *Sodium nitrate.*—Crystals as Cl-free as practicable to obtain 0.002% or less.
- (d) *Ferric indicator.*—Saturated. Use ferric potassium sulfate or ferric ammonium sulfate.

**26 DETERMINATION**

Weigh 10 g of fat into a 250 cc beaker and add 40 cc of alcohol and 5 cc of the KOH soln. Saponify the mixture and evaporate to dryness on the steam bath. Take up the soap in H<sub>2</sub>O (150 cc), warming if necessary. Cool, and make up to 250 cc.

Pipet 200 cc of the soln into a 500 cc Erlenmeyer flask. Close the flask with a stopper carrying a thermometer and having a small groove lengthwise in the side. Place the flask in a water bath maintained at about 80°. When the sample reaches about 80°, loosen the stopper and introduce 50 cc of the MgSO<sub>4</sub> soln from a pipet. Shake the flask with a rotary motion. Replace the stopper and thermometer and allow the flask to remain in the bath 8–10 min. longer at 70–80°, shaking the flask occasionally. Remove the flask and cool under the tap, with shaking, to 20–25°. Remove stopper and thermometer, stopper tightly, and shake vigorously 4 min. Allow the flask to stand in a bath at 20–25° until a water layer separates at the



bottom. Filter thru a Büchner funnel, removing all liquid possible by pressing with a horn spoon. Run a blank on cacao butter in the same manner.

Neutralize 200 cc of the filtrate until colorless to phenolphthalein with approximately 0.5 *N* H<sub>2</sub>SO<sub>4</sub> soln in a 250 cc volumetric flask. Add 20 g of the NaNO<sub>3</sub> soln and when dissolved add 22.5 of 0.2 *N* AgNO<sub>3</sub> soln. Make to mark and shake 3 min. Allow the flask to stand a short time and filter thru a folded filter. To 200 cc of the filtrate add 6 cc of the ferric indicator and 4 cc of 40% HNO<sub>3</sub>. Titrate with 0.1 *N* NH<sub>4</sub>SCN to first color change (reddish brown).

Calculate as follows:

Silver number (mg of silver used per g of fat) =  $(a - b) \times 2.107$ , in which

$a$  = 1.6  $\times$  cc of 0.2 *N* silver nitrate soln added; and

$b$  = cc of 0.1 *N* NH<sub>4</sub>SCN soln used in back titration.

$$\text{Factor } 2.107 = \frac{10.788 \text{ (mgAg per cc 0.1 } N \text{ soln)}}{5.12 \text{ (g of fat in aliquot titrated)}}$$

The silver number of palm kernel and coconut oils and stearins varies from about 26 for the stearins to 60 for whole coconut oil. Dairy butter gives a value of approximately 11.6, and cacao butter, 0.6.

## 27

CRUDE FIBER<sup>u</sup>—OFFICIAL

(For cacao products except milk chocolate.)

Treat 7 g of liquor or 50 g of sweet chocolate in a nursing bottle with two 100 cc portions of ether, centrifuging and decanting the supernatant liquor after each addition. Dry the residue in an oven at about 100° and then powder in the bottle with a flattened glass rod. (In some cases it may be necessary to grind the material in a mortar and extract a third time with ether.) Wash the mixture in the nursing bottle with three 100 cc portions of H<sub>2</sub>O at room temp., shaking well each time, until no cocoa material adheres to the bottle. Centrifuge after each washing for 10–15 min., and decant the aqueous layer. Wash the residue in the same fashion with two 100 cc portions of 95% alcohol and one 100 cc portion of ethyl ether. Transfer the residue to a Pt dish, dry to constant weight, and grind in a mortar. Weigh 2 g of the dried material and determine the percentage of crude fiber (*D*) as directed under XXVII, 27, using linen for both acid and alkaline filtrations. Calculate the percentage of crude fiber on moisture-, fat- and sugar-free basis (*E*) by the formula  $E = 0.7D$ .

## 28

## CRUDE FIBER IN MILK CHOCOLATE—OFFICIAL

Treat 50 g of milk chocolate with three 100 cc portions of ether in a nursing bottle, centrifuging and decanting the supernatant liquor after each addition. Dry the residue in the bottle and powder with a flattened glass rod. Shake with 100 cc of 1% Na<sub>2</sub>C<sub>2</sub>O<sub>4</sub> soln, and let stand 30 min. Centrifuge and decant the supernatant liquor. Wash in the nursing bottle with three 100 cc portions of distilled H<sub>2</sub>O at room temp., shaking well each time, until no cocoa material adheres to the bottle. Centrifuge after each washing for 10–15 min. and decant the aqueous layer. Wash the residue in the same fashion with two 100 cc portions of 95% alcohol and one 100 cc portion of ethyl ether. Transfer the residue to a Pt dish, dry to constant weight at 100°, and grind in a mortar. Weigh 2 g of the dried material and determine the percentage of crude fiber as directed in XXVII, 27. Use linen for both acid and alkaline filtrations. Percentage of crude fiber found  $\times 0.7$  = percentage of crude fiber on a true fat-, sugar-, moisture- and milk-free basis.

## STARCH

29

*I. Direct Acid Hydrolysis Method—Tentative*

Weigh 4 g of the sample if unsweetened, or 10 g if sweetened, into a small porcelain mortar; add 25 cc of ether and grind. After the coarser material has settled, decant the ether, together with the fine suspended matter, on an 11 cm paper of sufficiently fine texture to retain the crude starch. Repeat this treatment until no more coarse material remains. After the ether has evaporated from the filter, transfer the fat-free residue to the mortar by means of a jet of cold H<sub>2</sub>O and rub to an even paste, filtering on the paper previously used. Repeat this process until all the sugar is removed. In the case of sweetened products the filtrate should measure at least 500 cc. Determine crude starch in the extracted residue as directed under XXVII, 31.

30

*II. Diastase Method—Tentative*

Remove fat and sugar from 4 g of the sample if unsweetened, or 10 g if sweetened, as directed under 29. Carefully wash the wet residue into a beaker with 100 cc of H<sub>2</sub>O, heat to boiling over asbestos with constant stirring, and continue the boiling and stirring for 30 min. Replace the H<sub>2</sub>O lost by evaporation and immerse the beaker in a water bath kept at 55–60°. When the liquid has cooled to the temp. of the bath, add 20 cc of freshly prepared malt extract, XXVII, 32, and digest the mixture for 2 hours with occasional stirring. Boil a second time for 30 min., dilute, cool, and digest as before with another 20 cc portion of the malt extract. Heat again to boiling, cool, and transfer to a 250 cc flask. Add 3 cc of alumina cream, dilute to the mark, and filter thru a dry paper. The residue on the paper should show no signs of starch when examined microscopically. Continue from this point as directed under XXVII, 33, beginning with the words "Place 200 cc of the filtrate in a flask, add 20 cc of HCl (sp. gr. 1.125)."

31

## COLORING MATTERS—TENTATIVE

Proceed as directed under XXI, 2(e).

## SELECTED REFERENCES

- <sup>1</sup> J. Assoc. Official Agr. Chem., 16, 563 (1933); 17, 64 (1934).
- <sup>2</sup> Ibid., 16, 565 (1933); 17, 65 (1934).
- <sup>3</sup> Ibid., 16, 566 (1933); 17, 65, 379 (1934).
- <sup>4</sup> Ibid., 9, 46 (1926).
- <sup>5</sup> Ibid., 10, 42 (1927).
- <sup>6</sup> Ibid., 11, 45 (1928).
- <sup>7</sup> Ibid., 13, 45, 78, 486 (1930).
- <sup>8</sup> Ibid., 5, 263 (1921); 7, 150 (1923).
- <sup>9</sup> Ibid., 13, 43 (1930).
- <sup>10</sup> Ibid., 15, 549 (1932); 17, 64, 375 (1934).
- <sup>11</sup> Ibid., 14, 530 (1931); 16, 66 (1933).

## XX. CEREAL FOODS

### WHEAT FLOUR<sup>1</sup>

1

#### DIRECTIONS FOR SAMPLING—OFFICIAL

Sample a number of sacks equivalent to the square root of the number in the lot, but not less than 10, *i.e.*, 10 from 100 or less, 15 from 225, 20 from 400 sacks, etc.

Select the sacks to be sampled according to their exposure in the ratio of 4 from the most exposed, 3 from the next less exposed, 2 from the next, and 1 from the least exposed portion of the lot.

From each sack to be sampled, draw a core from one corner of the top diagonally to the center of the sack by means of a cylindrical, pointed, polished metal trier,  $\frac{1}{2}$  inch in diameter, with a slit at least  $\frac{1}{3}$  the circumference. Draw a second core from the other top corner to  $\frac{1}{2}$  the distance to the center of the sack.

Deliver the 2 cores at once to a clean, dry, air-tight container which has stood open for a few minutes near the lot of flour to be sampled and seal immediately. Use a separate container for each sack sampled. One of the following containers may be used: (1) A pint fruit jar provided with a rubber gasket; (2) a rubber pouch which can be tied or sealed to exclude moisture or air; (3) a tin can or box with a moisture and air-tight friction top.

Before opening the sample for analysis, alternately invert and roll each container 25 times, or more if necessary, to secure a homogeneous mixture. Avoid extreme temp. and humidities when opening the containers for analysis. Keep the sample tightly sealed at all other times.

#### TOTAL SOLIDS (MOISTURE, INDIRECT METHOD)

##### *Vacuum Oven Method<sup>2</sup>—Official*

2

#### APPARATUS

(a) *Metal dish*.—Diameter about 55 mm, height about 15 mm, provided with an inverted slip-in cover fitting tightly on inside.

(b) *Air-tight desiccator*.—Should contain reignited quick lime or  $\text{CaCl}_2$ .

(c) *Vacuum oven*.—Connect with a pump capable of maintaining a partial vacuum in the oven with a pressure equivalent to 25 mm or less of Hg and provided with a thermometer passing into the oven in such a way that the bulb is near the samples. Connect a  $\text{H}_2\text{SO}_4$  gas drying bottle with the oven for admitting dry air when releasing the vacuum.

3

#### DETERMINATION

Weigh accurately about 2 g of the well-mixed sample in a covered dish that previously has been dried at 98–100°, cooled in the desiccator, and weighed soon after attaining room temp. Loosen the cover (do not remove) and heat at 98–100° to constant weight (approximately 5 hours) in a partial vacuum having a pressure equivalent to 25 mm or less of Hg. Admit dry air into the oven to bring to atmospheric pressure. Immediately tighten the cover on the dish, transfer to the desiccator, and weigh soon after room temp. is attained. Report the flour residue as total solids and the loss in weight as moisture (indirect method).

4

*Air-Oven Method<sup>3</sup>—Official*

(Results closely approximate those obtained by the vacuum method.)

In a cooled and weighed dish (provided with a cover) that has been previously heated to approximately  $130^{\circ} \pm 3^{\circ}$ , weigh accurately approximately 2 g of the well-mixed sample. Uncover the sample and dry the dish, cover, and contents for an hour in an oven provided with an opening for ventilation and maintained at approximately  $130^{\circ} (\pm 3^{\circ})$ . Cover the dish while still in the oven, transfer to the desiccator, and weigh soon after room temp. is attained. Report the flour residue as total solids and the loss in weight as moisture (indirect method).

ASH<sup>4</sup>

5

*Method I.—Official*

Weigh 3–5 g of the well-mixed sample into a shallow, relatively broad ashing dish that has been ignited, cooled in a desiccator, and weighed soon after attaining room temp. Incinerate in a furnace at approximately  $550^{\circ}$  (dull red) until a light gray ash results, or until no further loss in weight occurs. Cool in the desiccator and weigh soon after room temp. is attained. Reignited quick lime or  $\text{CaC}_2$  is a satisfactory drying agent for the desiccator.

*Method II. Quick Ashing Method<sup>5</sup>—Tentative*

6

## SOLUTIONS

Dissolve in 40%  $\text{C}_2\text{H}_5\text{OH}$  sufficient of the nitrate chosen to yield upon ignition approximately 0.015 g of oxide per 10 cc.

1.9935 g  $\text{La}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}$  per liter yields 0.015 g  $\text{La}_2\text{O}_3$  per 10 cc.

1.8918 g  $\text{Ce}(\text{NO}_3)_6 \cdot 12\text{H}_2\text{O}$  per liter yields 0.015 g  $\text{CeO}_2$  per 10 cc.

1.5681 g  $\text{Th}(\text{NO}_3)_4 \cdot 4\text{H}_2\text{O}$  per liter yields 0.015 g  $\text{ThO}_2$  per 10 cc.

2.5441 g  $\text{Y}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}$  per liter yields 0.015 g  $\text{Y}_2\text{O}_3$  per 10 cc.

Run a blank on the nitrate soln to determine the exact quantity of oxide present.

7

## DETERMINATION

Weigh 3–5 g of flour into a dish approximately 65 mm in diameter and 25 mm in depth. Add with a pipet exactly 10 cc of the nitrate soln. Stir with a glass rod until all the flour is moistened. Clean the rod with a small piece of ashless filter paper and add the latter to the sample. Burn off excess alcohol. Transfer the dish to a muffle furnace that is already at  $850^{\circ}$ . Leave door of furnace open until flaming has ceased, then close. When the ash is entirely white (30–45 min.) remove the dishes to a desiccator, cool, and weigh. Weight of crude ash – weight of blank = true weight of ash.

ORIGINAL ASH OF PHOSPHATED AND SELF-RISING FLOUR<sup>6</sup>

8

*Gustafson Method*

To 20–25 g of the flour in a metal centrifuge tube (cup 2 in. in diameter, 6 in. deep), add sufficient  $\text{CCl}_4$  to fill the tube to within 1 in. of the top (about 250 cc). Centrifuge 5–7 min. at a speed of 1,600 r.p.m., and allow the centrifuge to come to rest slowly. Carefully skim off the flour, which is now in a compact layer on the surface of the  $\text{CCl}_4$ , with a large tablespoon, recovering as much of the flour as is possible in one spoonful. (With care, about 90% of the original flour may be recovered.) Allow the wet flour to dry overnight and proceed as directed under 5. (The carbon tetrachloride may be filtered, distilled, and used again.)

9

**TOTAL CARBON DIOXIDE IN SELF-RISING FLOUR<sup>a</sup>**

Use 17 g of flour and 40 cc of  $\text{H}_2\text{SO}_4$  (1+5) and proceed as directed under **XVII, 4-6**, as far as the calculation. Calculate as follows: Subtract the volume of acid used from the total buret reading and correct for temp. and pressure. Divide the corrected reading by 100 to obtain the percentage of  $\text{CO}_2$  (by weight) in the self-rising flour. To convert  $\text{CO}_2$  to  $\text{NaHCO}_3$ , multiply  $\text{CO}_2$  by the experimentally determined factor 2.01.

10

**CRUDE FAT OR ETHER EXTRACT—OFFICIAL**

Proceed as directed under **XXVII, 22**. With fine flour the addition of an equal weight of clean, dry sand may be necessary.

11

**FAT (ACID HYDROLYSIS METHOD)<sup>a</sup>—OFFICIAL**

Place 2 g of the flour in a 50 cc beaker, add 2 cc of 95% alcohol, and stir so as to moisten all particles. (The moistening of the sample with alcohol prevents lumping on addition of the acid.) Add 10 cc of  $\text{HCl}$  (25+11), mix well, set the beaker in a water bath held at 70–80°, and stir at frequent intervals for 30–40 min. Add 10 cc of 95% alcohol and cool. Transfer the mixture to a Röhrig or Mojonner fat extraction apparatus. Rinse the beaker into the extraction tube with 25 cc of ethyl ether in 3 portions and shake the mixture well. Add 25 cc of redistilled petroleum ether (b. p. below 60°) and mix well. Let stand until the upper liquid is practically clear. Draw off as much as possible of the ether-fat soln thru a filter consisting of a pledget of cotton packed just firmly enough in the stem of a funnel to allow free passage of the ether into a weighed 125 cc beaker-flask containing some porcelain chips or broken glass. Before weighing the beaker-flask dry it in a drying oven at 98–105° and then allow it to stand in the air to constant weight. Re-extract the liquid remaining in the tube twice, each time with only 15 cc of each ether. Shake well on the addition of each ether. Draw off the clear ether solns thru the filter into the same flask as before and wash the tip of the spigot, the funnel, and end of the funnel stem with a few cc of a mixture of the 2 ethers in equal volumes free from suspended  $\text{H}_2\text{O}$ . Evaporate the ethers slowly on a steam bath, then dry the fat in a drying oven at 90–105° until it ceases to lose weight (approximately 75 min.). Remove the flask from the oven, allow it to stand in the air until no further change in weight takes place, and weigh. (Owing to the size of the flask and the nature of the material, there is less error by cooling in air than in a desiccator.) Correct this weight by a blank determination on the reagents used.

12

**CRUDE FIBER—OFFICIAL**

Proceed as directed under **XXVII, 25-27**.

13

**ACIDITY OF WATER EXTRACT—TENTATIVE**

Weigh 18 g of the flour into a 500 cc Erlenmeyer flask and add 200 cc of  $\text{CO}_2$ -free  $\text{H}_2\text{O}$ . Keep the flask, loosely stoppered, for an hour in a water bath maintained at 40°, shaking occasionally. Filter thru a dry, folded filter, returning the first 10–15 cc of the filtrate to the filter. Titrate 100 cc of the clear filtrate with 0.05 *N*  $\text{NaOH}$  soln, using phenolphthalein indicator. 1 cc of 0.05 *N*  $\text{NaOH}$  soln = 0.05% acidity as lactic acid.

14

**HYDROGEN-ION CONCENTRATION<sup>a</sup>—OFFICIAL, FIRST ACTION**

Weigh 10 g of flour (or some multiple thereof) into a clean, dry Erlenmeyer flask and add for each 10 g of flour 100 cc of distilled  $\text{H}_2\text{O}$  at a temp. of 25°. Shake or

whirl the flask until the particles of flour are evenly suspended and the mixture is free from lumps. Place in a thermostat at 25° and shake, continuously or intermittently in such a manner as to keep the flour particles in suspension, for 30 min. Pour the extract into centrifuge tubes and whirl 5 min. Then pour the soln thru a hardened, dry, folded filter paper, discarding the first 5 cc and catching the remainder of the liquid in the colorimetric hydrogen-ion vessels. Immediately determine the hydrogen-ion concentration by comparison with suitable colorimetric standards.

15

## SUGARS—TENTATIVE

Determine reducing sugars and sucrose as directed under XXVII, 28, 29, and 30.

16

## PROTEIN—OFFICIAL

Determine N as directed under II, 21, 23, or 25, and multiply the percentage of N by 5.7 to obtain the percentage of protein. Use the factor 5.7 to convert N to protein in wheat used either for manufacturing purposes or for human food.

## 70 PER CENT ALCOHOL-SOLUBLE PROTEINS

17

*By Nitrogen Determination—Tentative*

Transfer 4 g of the flour to a 150–200 cc bottle or Erlenmeyer flask and add 100 cc of alcohol, 70% by volume, taking care that none of the material adheres to the bottom of the container. Shake thoroly 10–12 times at intervals of 30 min. at room temp., or shake continuously in a shaking machine for 1 hour, and then set aside overnight. Again shake thoroly and filter thru a dry, folded filter, returning the first runnings to the filter until a clear filtrate is obtained. Pipet 50 cc of the filtrate, equivalent to 2 g of the sample, into a Kjeldahl flask; dilute with 100 cc of H<sub>2</sub>O to prevent frothing during digestion; and determine N as directed under II, 21, 23 or 25. Make a blank determination on the reagents.

*By Polarization—Tentative*

18

## REAGENT

*Millon's reagent.*—Dissolve metallic Hg in an equal weight of HNO<sub>3</sub> and dilute the soln with an equal volume of H<sub>2</sub>O. A freshly prepared soln must be used.

19

## DETERMINATION

Weigh 15.97 g of the flour into a 300 cc flask and add 100 cc of alcohol (sp. gr. 0.90). Shake at 30 min. intervals for 3 hours and let stand overnight. Filter thru a dry, folded filter and polarize in a 200 mm tube. Precipitate the proteins in 50 cc of the filtrate by the addition of 5 cc of Millon's reagent. Shake, filter, and polarize the filtrate in a 200 mm tube. Multiply the reading in degrees Ventzke by 1.1 to correct for the dilution and deduct the product from the first reading. This difference, multiplied by 0.2, gives the percentage of gliadin N.<sup>9</sup>

## 20 PROTEINS SOLUBLE IN 5 PER CENT POTASSIUM SULFATE SOLUTION—TENTATIVE

Weigh 6 g of the flour into a 200 cc flask and introduce exactly 100 cc of 5% K<sub>2</sub>SO<sub>4</sub> soln. Shake at 30 min. intervals for 3 hours or, better, agitate at moderate speed in a mechanical shaker for 1 hour; let settle 30 min.; and filter. Determine the N in 50 cc of the filtrate as directed under II, 23 or 25, making allowance for the N contained in the reagents.

**21 GLOBULIN AND ALBUMIN (EDESTIN AND LEUCOSIN) AND AMINO NITROGEN—TENTATIVE**

Weigh 10 g of the flour into a 500 cc Erlenmeyer flask, add 250 cc of 1% NaCl soln, stopper the flask, and shake thoroly. Let stand, with occasional shaking, for 3 hours; filter; and evaporate 100 cc of the filtrate to a small volume in a Kjeldahl digestion flask with 5 cc of  $\text{H}_2\text{SO}_4$ . Add 25 cc more acid and determine the N as directed under 21, 23, or 25. To a second 100 cc of the filtrate add 5 cc of 20% phosphotungstic acid soln, shake thoroly, allow to settle, and filter by decantation. Wash slightly with  $\text{H}_2\text{O}$ , concentrate the filtrate with 5 cc of  $\text{H}_2\text{SO}_4$  in a Kjeldahl flask, and determine the amino N as directed under II, 21, 23, or 25. Deduct the amino N from N found in the first fraction to obtain the N as globulin and albumin.<sup>10</sup> Make allowance for the N contained in the reagents.

**GLUTENIN**

22

*Method I.—Tentative*

Deduct the sum of the  $\text{K}_2\text{SO}_4$ -soluble N, 20, and the alcohol-soluble N, 17, from the total organic and ammoniacal N, 16, and multiply the difference by 5.7.

23

*Method II.—Tentative<sup>11</sup>*

(Flour and reagents should be allowed a minimum exposure to the air at all times.)

Weigh 8 g of flour into a 200 cc flask, preferably a sugar flask or one that readily permits thoro mixing of the suspension when shaken. Add 0.2 g of freshly powdered  $\text{Ba}(\text{OH})_2$ , follow at once with 50 cc of distilled  $\text{H}_2\text{O}$  ( $\text{CO}_2$ -free), and stopper tightly. Shake immediately to form a smooth suspension. Let stand for 1 hour at room temp., shaking frequently. Add sufficient 96% methyl alcohol free from acids, aldehydes, and ketones (synthetic methanol preferred) to allow 5 cc of liquid above the mark (to correct for volume of flour) when thoroly mixed. Shake vigorously for 2 min. After the starch settles to the bottom, pour the supernatant liquid *at once* thru a cotton plug, repeating the filtrations 2 or 3 times if necessary. Immediately withdraw 50 cc for a Kjeldahl N determination, II, 17. Do not allow more than 15 min. to elapse from the time the methyl alcohol is added to the withdrawal of the 50 cc aliquot, because gliadin will begin to precipitate after standing for a short period of time. To prevent troublesome foaming add 150–200 cc of  $\text{H}_2\text{O}$  to the Kjeldahl flask before starting the digestion of the alcoholic extract. Convert the N to protein by the factor 5.7, subtract the percentage of protein in the extract from the percentage of total protein ( $\text{N} \times 5.7$ ) as determined in a separate portion of flour, and record the difference as the percentage of glutenin in the flour.

**CRUDE GLUTEN**

24

*Qualitative Test<sup>12</sup>—Tentative*

Place a very small quantity of the flour (about 1.5 mg) on a microscope slide; add a drop of  $\text{H}_2\text{O}$  containing 0.2 g of water-soluble eosin in 1 liter; and mix by means of a cover-glass, holding it at first in such a manner that it is raised slightly above the slide and taking care that none of the flour escapes from beneath it. Finally allow the cover-glass to rest on the slide and rub it back and forth until the gluten has collected into rolls. Carry out the operation on a white paper so that the formation of the gluten rolls can be easily noted. Wheat flour, or other flours containing gluten, show by this treatment a copious quantity of gluten, which absorbs the eosin with

avidity, assuming a carmine color. Rye flour and corn flour yield only a trace of gluten; buckwheat flour, no appreciable quantity. If the flour is coarse or contains a considerable quantity of bran elements, as is true of buckwheat flour and low-grade wheat flour, make the test after bolting, as the bran particles and coarse lumps interfere with the formation of gluten rolls.

*Quantitative Method—Tentative*

25

(Results are approximate.)

Weigh 25 g of the flour into a cup or porcelain mortar; add sufficient tap  $H_2O$  (about 15 cc) to form a firm dough ball; and work into a dough with a spatula or pestle, taking care that none of the material adheres to the utensil. Allow the dough to stand in  $H_2O$  at room temp. for an hour; knead gently in a stream of tap  $H_2O$  until the starch and all soluble matters are removed. Do this operation, which requires approximately 12 min., over bolting cloth. To determine whether or not the gluten is starch-free let 1 or 2 drops of the wash  $H_2O$ , obtained by squeezing the gluten, fall into a beaker containing perfectly clear  $H_2O$ . If starch is present, a cloudiness appears. Allow the gluten thus obtained to stand in  $H_2O$  for an hour, press as dry as possible with the hand, roll into a ball, place in a weighed flat-bottomed dish, and weigh as moist gluten. Transfer to an oven, dry to constant weight at  $100^\circ$  (about 24 hours), cool, and weigh as dry gluten; or heat the moist gluten at approximately  $230^\circ$  for 15–20 min., or until the puffed gluten ball has become firm. Dry to constant weight in a drying oven.

**26 WATER-SOLUBLE PROTEIN-NITROGEN PRECIPITABLE BY 40 PER CENT ALCOHOL<sup>1a</sup>—TENTATIVE**

Place 20 g of the sample in an 8 oz nursing bottle, add 100 cc of  $H_2O$  from a pipet, shake the bottle to prevent lumping of the sample, and add exactly 100 cc more  $H_2O$ . Mix the contents of the stoppered bottle gently by hand or on a slowly revolving wheel for 1 hour. (The temp. of the  $H_2O$  should not exceed  $30^\circ$ .) Centrifuge to facilitate filtration and filter thru a thin pad of ignited asbestos (fine) in a Hirsch funnel, using light suction. Determine N in 50 cc of the filtrate as directed under II, 21, 23, or 25, distilling the  $NH_3$  into 20 cc of 0.1 *N* acid. Run a blank on the reagents. Pipet off 100 cc of the above filtrate into a 200 cc volumetric flask, add 15 cc of NaCl soln (28 g diluted to 300 cc), fill nearly to the mark with 95% alcohol, mix carefully to avoid foaming, cool to room temp., make up to the mark with alcohol, mix well, and allow to stand overnight. Pipet off the supernatant liquid and filter thru an 18½ cm fluted filter paper. Determine N in 100 cc of the filtrate as above. (In order to avoid bumping, it is advisable to add the  $H_2SO_4$  and boil off the alcohol before adding the  $K_2SO_4$  and  $HgO$ .) Subtract the value obtained from the water-soluble N to obtain the water-soluble N precipitable by 40% alcohol.

27

**LIPIDS<sup>1a</sup>—OFFICIAL**

Add 15 cc of alcohol, 70% by volume, to 5 g of the flour in a 200 cc nursing bottle. Give the bottle a gentle rotary motion so as to moisten all the particles with the alcohol, stopper, and set in a water bath kept at  $75$ – $80^\circ$ . Heat for 15 min. with frequent mixing by the same rotary motion. Add 27 cc of 95% alcohol, stopper the bottle, and shake vigorously for 2 min. Cool, add 45 cc of ether, and shake well for 5 min. (The sample should now be in a fine state of division.) Centrifuge just sufficiently to throw the solid particles out of suspension but not so as to pack the sample too firmly. Decant the liquid into a 250 cc beaker containing some bits of broken



porcelain or glass, and rinse off the bottle neck with ether. Re-extract the sample with 3 successive 20 cc portions of ether, shake 1 or 2 min. each time, centrifuge, and decant into the beaker containing the first extract. Evaporate the combined ether-alcohol extracts just to dryness on the steam bath. Drive off any remaining moisture on the sides of the beaker by placing in a drying oven at 98–105° for 5 min. Dissolve the dry extract in approximately 15 cc of  $\text{CHCl}_3$  and filter the soln into a previously dried and weighed Pt dish thru a pledget of cotton packed in the stem of a funnel. Free with a glass rod any solid extract adhering to the beaker and transfer thru the filter into the first washings by means of  $\text{CHCl}_3$  from a wash bottle all extract from the beaker bottom and sides. Finally wash the funnel and stem tip. The filtrate should be perfectly clear. Evaporate the  $\text{CHCl}_3$  on a steam bath and dry the dish and contents in a drying oven at 98–105° until no more weight is lost (75–90 min.). Weigh. Report the extract as lipoids.

## 28 LIPOID PHOSPHORIC ACID<sup>18</sup> ( $\text{P}_2\text{O}_5$ )—OFFICIAL

Dissolve the lipoids in 5–10 cc of  $\text{CHCl}_3$ , add 5–10 cc of 4% alcoholic KOH soln, evaporate to dryness on a steam bath, and char well in a furnace at a faint red heat. Cover the dish with a cover-glass, add sufficient  $\text{HNO}_3$  (1+9) to make the soln slightly acid, warm on a steam bath, and filter. Wash the residue and filter well with hot  $\text{H}_2\text{O}$ . Determine  $\text{P}_2\text{O}_5$  in the filtrate as directed under II, 9 or 12. Report as lipid  $\text{P}_2\text{O}_5$ .

## UNSAPONIFIABLE RESIDUE<sup>14</sup>

### 29

#### *Modified Kerr-Sorber Method<sup>17</sup>—Tentative*

Place the extract from 5 g of flour prepared as directed under 27 in a 100–200 cc saponification flask. Add 3 cc of KOH soln (100 g KOH in 100 cc  $\text{H}_2\text{O}$ ). Place a small, short-stemmed funnel in the neck of the flask to serve as a condenser. Boil gently on the steam bath for about 20 min., or until complete saponification occurs. Cool to about 30°, add 50 cc of ether, mix, and transfer to a 500 cc separatory funnel. Rinse the flask with 2 successive 50 cc portions of ether, add to the separatory funnel, and mix thoroly. Wash the saponification flask with 100 cc of an approximately 0.2 N KOH soln (11.2 g dissolved in 1000 cc of  $\text{H}_2\text{O}$ ) and pour into the separatory funnel in a slow, steady stream. Rotate the funnel very gently to secure better contact of the solns but do not shake, because shaking at this stage produces a stubborn emulsion. Allow the liquids to separate completely and slowly draw off as much of the soap soln as possible. Do not draw off any layer of emulsion that may be formed. Keep the volume of the ether at about 150 cc by replacing that dissolved by the wash solns. Further treat the ether soln with 2 successive 100 cc portions of the dilute KOH soln in the manner described previously. Add 30 cc of  $\text{H}_2\text{O}$  to the ether and rapidly rotate the liquid layers. When the layers have separated completely, draw off the  $\text{H}_2\text{O}$ , repeating this treatment until the washings are free from alkali, as shown by testing with phenolphthalein (3 washings usually suffice). Transfer the ether soln quantitatively thru a pledget of cotton in the stem of a funnel to a weighed 250 cc Erlenmeyer or beaker flask. Before weighing the flask dry it in an oven at about 100°, and then allow it to stand in the air to constant weight. Distil off the ether and dry the flask and residue at about 100° until no further loss in weight occurs. Allow the flask with unsaponifiable matter to come to equilibrium with the atmosphere before weighing. Deduct from the weight of the unsaponifiable matter any blank obtained from the reagents used.

30

## EXTRACT SOLUBLE IN COLD WATER—TENTATIVE

Weigh 20 g of the flour into a 500 cc Erlenmeyer flask and add gradually 200 cc of H<sub>2</sub>O at a temp. not higher than 10°. Shake vigorously when about 50 cc of H<sub>2</sub>O has been added and continue shaking during the addition of the remaining H<sub>2</sub>O. Allow to stand at 10° for 40 min., shaking occasionally. Filter rapidly, returning the first runnings to the filter, until a clear filtrate is obtained. Pipet 20 cc of the clear filtrate into a weighed dish, evaporate to dryness on a steam bath, and dry to constant weight in an oven at about 100° for periods of 30 min.

STARCH<sup>18</sup>—TENTATIVE

31

## REAGENT

*Hydrochloric acid.*—Mix approximately equal volumes of HCl and H<sub>2</sub>O and adjust by titration so that 100 cc of this soln contains 20.5–21.0 g of HCl.

32

## DETERMINATION

Weigh accurately a sufficient quantity of finely ground sample (should readily pass thru a 20-mesh sieve) to represent 0.5–1.0 g of starch. Transfer to a funnel fitted with a 9 cm S & S No. 589 white ribbon or Whatman No. 40 filter paper and extract by nearly filling the filter 4 times with ethyl ether; likewise extract with alcohol (70% by volume) and with H<sub>2</sub>O. Allow to drain 1 hour uncovered. Transfer the drained filter and contents to a 50 cc beaker. In the next step use a stirring rod having a flattened button-like end 15 mm in diameter, and (very important) tamp with a twisting motion during the time specified in order to get the filter paper completely disintegrated and thus insure the complete suspension of the starch in the HCl soln but not to hydrolyze any of it. Complete the maceration while there is a small amount of HCl present and the whole contents is a rather thick paste. (If this optimum condition is obtained practically duplicate results will follow.) Add the HCl reagent at 15° to the beaker containing the sample, using a fast delivering 10 cc Mohr pipet with 1 cc marked off at the lower end with heavy pencil marks. Keep the acid supply on the bench, but do not allow it to get above 18°.

Proceed as follows, adding the HCl in the quantities given: Add 1 cc, tamp 1 min.; add 1 cc, tamp 2 min.; add 1 cc, tamp 2 min.; add 1 cc, tamp 1 min.; add 1 cc, tamp 1 min.; add 1 cc, tamp 1 min.; add 1 cc, tamp 1 min.

Fill the beaker half full with the acid and stir 30 sec. Fill beaker  $\frac{3}{4}$  full and stir 30 sec. (In 10 min. during this treatment the paper should be completely disintegrated and in a smooth state of suspension, the tamping should be continued vigorously during this time, and as little time as possible should be spent adding the acid.) Immediately transfer to a 100 cc wide-mouthed volumetric flask, rinsing out the beaker with the HCl; carefully make to volume with the HCl reagent and add 0.5 cc for volume of filter paper (this step requires 2 min.). Shake the stoppered flask vigorously for 5 min, and allow to stand 5 min. in a beaker of H<sub>2</sub>O at 20°. Shake twice and filter immediately into a 250 cc suction flask thru a small Büchner funnel (41 mm in diameter) fitted with a thin layer of asbestos and filled half full with dry, fluffy asbestos. (The filtration requires 1 min. only.) Immediately pipet 50 cc of the filtrate into a 200 cc beaker (tall form) containing 115 cc of 95% alcohol. (The quantity of starch finally weighed will then vary from 0.25–0.5 g. The time consumed from the initial addition of the acid is 24 min.) Allow the pipet to drain completely and then stir with a whipping motion for 1 min. to flocculate the precipitated starch. Wash down the sides of the beaker with 70% alcohol. Allow

to stand 3–4 min., until nearly all the precipitate has settled, and then carefully decant the supernatant liquid, which is somewhat turbid, so that little or no precipitate passes into the weighed Gooch crucible, which has been fitted with a thin pad of ignited asbestos and is half filled with fluffy ignited asbestos. Wash the precipitate, and filter by decantation, using successively two 40 cc portions of 70% alcohol (by volume), then 4 times, using about 30 cc portions of 95% alcohol, each time breaking up the precipitate by rapid stirring and allowing the precipitate to settle before decantation. After each stirring rinse the sides of the beaker with a small stream of alcohol to prevent the starch from drying and sticking. Finally transfer the starch completely by means of a jet of 95% alcohol and wash the sides of the Gooch and precipitate with a little of the alcohol. (All these filtrations are very fast.) Dry the crucible and contents uncovered for 2 hours at 130°; cover the crucible immediately and place in a desiccator charged with  $P_2O_5$ , fresh  $H_2SO_4$ , or freshly ignited  $CaO$ ; cool 10 min. and weigh. Multiply the result by 2 and report as starch. Caution: To obtain satisfactory results, these directions must be followed carefully in every detail. As the steps are timed it is essential to learn the procedure so that no time will be lost in following it thru. Arrange everything needed in the determination before the  $HCl$  is added to the sample.

#### CHLORINE

##### 33 *Qualitative Test (Chlorine-Bleached Flours)—Tentative*

Extract 30 g of the flour with gasoline and allow the solvent to evaporate. A small quantity of oil remains. Heat a piece of  $Cu$  wire in a colorless gas flame until it is black and no longer colors the flame green. Dip the hot end of the wire into the oil and again bring into the flame. If  $Cl$  or  $Br$  has been used as a bleaching agent, a green or blue coloration is produced.

##### *Quantitative Method I.<sup>19</sup>—Tentative*

##### 34

#### REAGENTS

(a) *Petroleum ether*.—Fractionated at 60–100°. Avoid low-boiling petroleum ether because of errors introduced thru rapid evaporation.

(b) *Alcoholic sodium hydroxide soln.*—Dissolve 40 g of  $NaOH$  in 1 liter of 95% alcohol.

(c) *Potassium chromate indicator*.—Dissolve 5 g of  $K_2CrO_4$  in  $H_2O$ , add the  $AgNO_3$  soln (d) until a slight red precipitate is produced, filter, and dilute to 100 cc.

(d) *Standard silver nitrate soln.*—Dissolve 4.791 g of  $AgNO_3$  in  $H_2O$  and dilute to 1 liter. 1 cc = 1 mg of  $Cl$ . Check by titration against a standardized soln of  $NaCl$ .

##### 35

#### DETERMINATION

Weigh 75 g of the flour into a cork-stoppered bottle and add from a pipet 150 cc of the petroleum ether. Stopper tightly and shake vigorously for about 1 min. Allow to stand 1 hour, again shake until the flour particles are in suspension, and set aside overnight. Shake once more to suspend the flour particles, allow to settle for a few min., and filter thru a dry folded filter. (The funnel and receiving flask should be covered to reduce evaporation during filtration.) Pipet 50 cc of the filtrate into a  $Pt$  dish of about 80 cc capacity. If a  $Pt$  dish of this size is not available, evaporate the 50 cc of filtrate to a small volume in a porcelain evaporating dish on a steam bath and carefully transfer the fatty concentrate to a small  $Pt$  dish, washing out the last traces of fat with several portions of petroleum ether. Add 5 cc of the alcoholic

NaOH soln and evaporate to dryness on a steam bath. Char carefully in a muffle at low redness. Extract the charred mass with 2 successive 20 cc portions of  $\text{HNO}_3$  (1+3), being careful to avoid mechanical losses due to evolution of  $\text{CO}_2$ . Filter these extracts thru a 7 cm quantitative filter paper into a 300 cc flask. Extract the mass 2 or 3 times with  $\text{H}_2\text{O}$ , filtering each portion thru the same 7 cm paper. Return this filter paper to the Pt dish containing the charred residue and ignite to a white ash in a muffle furnace. Dissolve the ash in  $\text{HNO}_3$  (1+16) and add to the soln already obtained. Neutralize the acidity with a slight excess of dry  $\text{CaCO}_3$ , add 5 cc of the  $\text{K}_2\text{CrO}_4$  indicator, and titrate with the standard  $\text{AgNO}_3$  soln. At the same time and under the same conditions prepare and conduct a blank containing the quantity of all reagents used in the determination. Since  $\text{CaCO}_3$  commonly contains appreciable quantities of chlorides, a definite weighed quantity of this reagent should be used in each determination and the same quantity used in the blank. Correct the buret reading by the number of cc of the standard  $\text{AgNO}_3$  soln required to give in the blank the shade obtained at the end of the titration of the sample, using in both sample and blank 5 cc of the  $\text{K}_2\text{CrO}_4$  indicator. Report results in parts of Cl per million of flour.

Since the quantity of Cl involved in this determination is relatively small, care should be taken to insure that the laboratory atmosphere is as free from Cl as possible.

#### *Quantitative Method II.<sup>20</sup>—Tentative*

#### 36

##### REAGENTS

- (a) *Alcohol*.—70%. Mix 73 volumes of 95% alcohol with 27 volumes of  $\text{H}_2\text{O}$ .
- (b) *Alcoholic soda soln*.—To 95% alcohol add metallic Na cut into small pieces in the proportion of 40 g of Na to 1000 g of alcohol.
- (c) *Ferric-ammonium alum soln*.—To a cold saturated soln of ferric-ammonium alum add enough  $\text{HNO}_3$  to cause the disappearance of the brown color.

#### 37

##### DETERMINATION

Weigh 20 g of flour into a 500 cc Erlenmeyer flask, add 60 cc of 70% alcohol, place the flask upon a steam bath, and heat gently ( $\text{H}_2\text{O}$  should steam but not boil), at the same time rotating the flask until the flour and liquid form a uniform mixture. Add 60 cc of 95% alcohol. Stopper the flask and shake thoroly for 2 min. Allow to cool. Add 75 cc of ethyl ether and shake the flask thoroly, then add 150 cc of petroleum ether, and again shake the flask thoroly. Pour the entire liquid contents into a separatory funnel, being careful to avoid, so far as possible, transference of any flour particles. Add to the flask containing the flour, 40 cc of petroleum ether, shake thoroly, and pour the contents into the separatory funnel. Repeat with another 40 cc portion of petroleum ether. Wash the solvents twice with  $\text{H}_2\text{O}$ , using 30 cc of  $\text{H}_2\text{O}$  the first time and 10–12 cc the second time; shake thoroly each time, and allow to stand until two sharply defined layers of liquid are formed. Run the washed solvents into a large evaporating dish (or beaker), add 10 cc of the alcoholic soda soln, and evaporate to about 10–15 cc. Pour this liquid into a 50 cc Pt dish and wash out the evaporating dish with small portions of 95% alcohol until all the liquid and residue have been transferred to the Pt dish. Evaporate the contents of the dish to dryness on the steam bath and place the dish with the residue over a small yellow flame of a Bunsen burner. Char the residue but do not heat even to low redness because the alkali may react with the Pt. Allow to cool and add a small quantity of

H<sub>2</sub>O and 5 cc of HNO<sub>3</sub> (2+1). Boil, and then pour thru an ashless filter paper (12½ cm in diameter), catching the filtrate in a sugar flask calibrated at 100 cc and 110 cc. Again boil the residue with a small quantity of H<sub>2</sub>O and filter. Remove the filter paper, fold once and place in a Pt dish, and heat to low redness until practically all the paper and residue have been reduced to a gray ash, applying low heat to prevent volatilization of chlorides. Add a small quantity of H<sub>2</sub>O and 2.5 cc of HNO<sub>3</sub>, boil, and filter. Again boil once or twice with small quantities of H<sub>2</sub>O, filtering as before. Add to the liquid in the sugar flask 25 cc of 0.005 N AgNO<sub>3</sub> soln, add H<sub>2</sub>O to bring the liquid approximately to the 100 cc mark, and place the flask in boiling H<sub>2</sub>O for about 5 min. Remove, and allow to cool to room temp. Bring the liquid exactly to the 110 cc mark by adding H<sub>2</sub>O, stopper the flask, and mix the contents well. Filter thru a dry, fine-pore filter paper (12.5 cm in diameter), and return the first portion of the filtrate to the original soln. Continue to refilter until the filtrate is entirely clear, and thus secure 100 cc of filtrate. Transfer the entire 100 cc to a white porcelain casserole, add 2 cc of the ferric-ammonium alum soln, and titrate with 0.005 N KCNS until a permanent light brown coloration appears. Deduct the blank determined on all the reagents used and calculate results to the dry basis.

#### NITRITE NITROGEN—TENTATIVE

##### 38

##### REAGENTS

(a) *Sulfanilic acid soln.*—Dissolve 0.5 g of sulfanilic acid in 150 cc of 20% acetic acid.

(b) *Alpha-naphthylamine hydrochloride soln.*—Dissolve, by heating, 0.2 g of the salt in 150 cc of 20% acetic acid.

(c) *Standard nitrite soln.*—Dissolve 0.1097 g of dry AgNO<sub>3</sub> in about 20 cc of hot H<sub>2</sub>O, add 0.10 g of NaCl, shake until the AgCl flocculates, and dilute to 1 liter. Draw off 10 cc of the clear soln and dilute to 1 liter. 1 cc of the last soln = 0.0001 mg of N as nitrite, XXXVII, 14(c).

The AgNO<sub>3</sub> may be prepared as follows: To a cold soln of about 2 g of NaNO<sub>2</sub> or KNO<sub>2</sub> in 50 cc of H<sub>2</sub>O, add a soln of AgNO<sub>3</sub> so long as a precipitate forms. Decant the liquid and thoroly wash the precipitate with cold H<sub>2</sub>O. Dissolve in boiling H<sub>2</sub>O. (On cooling, the AgNO<sub>3</sub> crystallizes out.) Dry the crystals in the dark at ordinary temp. (preferably in a vacuum).

##### 39

##### DETERMINATION

(1) Select a series of 100 cc volumetric flasks of uniform dimensions and color and place 2 g of high-grade, nitrite-free flour in each flask; add approximately 70 cc of nitrite-free H<sub>2</sub>O and shake until the flour is thoroly moistened. Add to these flasks varying quantities of the standard NaNO<sub>2</sub> soln, so that a series of comparison standards will be obtained having a range covering the probable nitrite content of the unknown sample. Reserve one flask for a blank test. In order to avoid making a large series of standards it is well to make a preliminary test to ascertain the approximate nitrite content of the unknown. If the quantity of nitrite present is small, the nitrite soln in the flasks may be increased by 0.4 cc each. If bleaching is excessive, 1 g of flour may be used thruout, or the standards may be given a wider variation in nitrite content.

To each of 2 similar flasks add 2 g of the flour and 90 cc of H<sub>2</sub>O; shake thoroly; digest all the flasks, including the blank, in a water bath at 40° for at least 15 min.; and add 2 cc each of the sulfanilic acid and alpha-naphthylamine hydrochloride solns to each flask, shaking the mixture after the addition of each reagent. Continue the digestion at 40° for an additional 20 min. (The color must be developed in all

the flasks under conditions as nearly uniform as possible.) Make up to the marks with nitrite-free  $\text{H}_2\text{O}$  and compare the unknown with the series of standards. This may be done in a large, white-enameled pan, the effect of the turbidity due to the flour being minimized by the white background. The solns should be allowed to subside and should not be shaken during comparison; or,

(2) Weigh 20 g of the flour into a 500 cc Erlenmeyer flask; add 200 cc of nitrite-free  $\text{H}_2\text{O}$ , previously warmed to  $40^\circ$ ; and close the flask with a rubber stopper. Shake vigorously for 5 min. and digest for 1 hour in a water bath, keeping the temp. of the liquid in the flask at  $40^\circ$  and shaking at 10 min. intervals. Finally filter thru a nitrite-free filter. Return the first runnings to the filter until a clear filtrate is obtained. Pipet 50 cc of the filtrate and 50 cc of the standard nitrite soln into small flasks; add to each 50 cc of  $\text{H}_2\text{O}$  and 2 cc each of the sulfanilic acid and alpha-naphthylamine hydrochloride solns; shake; and allow to stand 1 hour to bring out the color. Compare the two solns in a colorimeter. Divide the height of the column of the standard soln by that of the soln of the sample to obtain the parts of nitrous N (free and combined) per million of flour.

#### 40

#### BENZOYL PEROXIDE BLEACH IN FLOUR<sup>21</sup>—TENTATIVE

Add a mixture of 125 g of flour, 100 g of salt, and 80 g of  $\text{CaCl}_2$  (dried) to a short-necked 800 cc Kjeldahl flask which contains 250 cc of  $\text{H}_2\text{O}$  and 30 cc of  $\text{HCl}$  and shake vigorously. Immediately connect for steam distillation and distil 325 cc as rapidly as possible after initial foaming. Saturate the distillate with 100 g of salt, transfer to a 500 cc separatory funnel, and extract with 50 cc of ethyl ether. Again extract the salt soln with 50 cc of ethyl ether and discard the salt soln. Pour the ether from the two funnels into a large, flat crystallizing dish and evaporate at room temp. with the aid of an electric fan. Dissolve the residue in 5 cc of acetone, add 7 cc of 2 *N*  $\text{NaOH}$ , and transfer to a 150 cc beaker. Rinse the crystallizing dish into the beaker with 35–40 cc of  $\text{H}_2\text{O}$ . Heat over a flame, carefully at first, then boil about 20 min. until all the acetone is removed. Add  $\text{H}_2\text{O}$  occasionally to keep the volume approximately constant. While hot transfer to a separatory funnel and add 25 cc of amyl alcohol. Pour the lower layer into a 250 cc separatory funnel and extract again with 20 cc of amyl alcohol. Pour the lower layer into a 250 cc casserole. Combine the amyl alcohol solns, add an equal volume of petroleum ether, and extract 3 times with 5 cc of  $\text{H}_2\text{O}$ , and add to the aqueous soln in the casserole. Discard the amyl alcohol-ether soln. Add 2 cc of superoxol (30% hydrogen peroxide) to the aqueous soln. Bring to boiling slowly and boil until foaming ceases. Cool. Make acid to litmus with  $\text{H}_2\text{SO}_4$  (1+1). Pour into a small separatory funnel. Cool, and extract twice with 20 cc of a mixture of equal parts of ethyl ether and petroleum ether. Pour the combined ether extract into a large test tube, add 2 cc of 2 *N*  $\text{NaOH}$ , stopper the tube, and shake. Place a thread in the tube to insure even boiling and evaporate slowly at first, by holding over steam. Place the tube in a vigorously boiling saturated salt soln. Add a drop of superoxol and when the foaming ceases add another drop. Continue adding a drop at a time until the soln is almost colorless. Add a drop or two of  $\text{H}_2\text{O}$  occasionally if the evaporation is too rapid. Evaporate completely to dryness and heat at  $100^\circ$  in a vacuum for about 30 min. Cool, and add 0.3 g of  $\text{KNO}_3$  and 3 cc of  $\text{H}_2\text{SO}_4$ . Heat in a boiling water bath for 20 min., taking care to get all the solid material into soln. (The aid of a stirring rod is essential.) Cool the tube in cold  $\text{H}_2\text{O}$  and add 6 cc of  $\text{H}_2\text{O}$  with stirring. When cool, add 15 cc of  $\text{NH}_4\text{OH}$  slowly with continuous stirring to keep the soln cool. Add 2 cc of 6% hydroxylamine hydrochloride soln and place in a  $65^\circ$  water bath for 5–6 min., stirring occasionally.

Cool in cold  $H_2O$ , filter into another similar tube, and observe the color of the filtrate. A red color indicates the presence of benzoic acid.

To make this method semiquantitative proceed as follows:

Prepare a series of standard tubes containing 0.2–1.5 mg of benzoic acid in ether soln (1 mg to 1 cc). Add 2 cc of 2 *N* NaOH to each. Mix by shaking and proceed exactly as with the sample, starting, "place the tube in a vigorously boiling saturated salt soln." Comparison of the sample with the standards familiarizes the analyst with the color to be expected and offers an approximate estimation of the amount of benzoic acid recovered. For the calculation of the approximate amount of benzoic acid in p.p.m. multiply the sample reading in mg by 32. This factor is based on a 125 g sample and a minimum recovery of 25% benzoic acid.

#### 41 GASOLINE COLOR VALUE—TENTATIVE

Place 20 g of the flour in a wide-mouthed, glass-stoppered 120 cc bottle and add 100 cc of colorless gasoline. Stopper tightly and shake vigorously for 5 min. Allow to stand 16 hours, shake again for a few seconds until the flour has been loosened from the bottom of the bottle and thoroly mixed with the gasoline, then filter immediately thru a dry 11 cm paper into an Erlenmeyer flask, keeping the funnel covered with a watch-glass to prevent evaporation. In order to secure a clear filtrate, allow a certain quantity of the flour to pass over into the filter, and pass the first portion of the filtrate thru a second time. (It will be found convenient to fit the filter paper to the funnel by means of  $H_2O$  and to dry thoroly either by standing overnight in a well-ventilated place or by heating.)

Determine the color value of the clear gasoline soln in a Schreiner or similar colorimeter, using for comparison a 0.005%  $K_2CrO_4$  soln. This soln corresponds to a gasoline number of 1.0 and is conveniently prepared by diluting 10 cc of a 0.5% soln to 1 liter. Adjust the colorimeter tube, containing the gasoline soln, to read 50 mm; then raise or lower the tube containing the standard chromate soln until the shades of yellow in both tubes match. The reading of the chromate soln, divided by the reading of the gasoline soln = the gasoline color value. The color value may also be determined in Nessler tubes by using for comparison  $K_2CrO_4$  solns of various dilutions prepared from a 0.5% soln and filling the tubes in all cases to the height of 50 mm.

#### DETECTION OF RYE FLOUR IN WHEAT FLOUR<sup>21</sup>

##### 42 *Chloroform Test—Tentative*

(a) *In ordinary flours.*—To 10 g of flour in a test tube, add 20 cc of  $CHCl_3$ , stopper the tube, and shake well. Allow the tube to stand in a vertical position until the heavier particles have settled out, preferably overnight. If rye is present, the sediment in the tube will be of greenish or bluish tint. Wheat gives a yellowish sediment.

Make comparisons with wheat and rye flours of known purity and with mixtures of varying proportions, such as 5, 10 and 15%, etc., of rye.

(b) *In phosphated flours.*—Treat flours containing phosphate or leavening agents with  $CCl_4$  in a separatory funnel to remove added salts. After removing the sediment of salts from the separatory funnel collect the flour on a filter, transfer to a test tube, and treat with  $CHCl_3$ .

#### DIASTATIC ACTIVITY OF FLOUR<sup>22</sup>—OFFICIAL

##### 43

##### REAGENTS

(a) *Buffer soln.*—Make up 3 cc of glacial acetic acid and 4.1 g of *anhydrous* sodium acetate to 1 liter with  $H_2O$ . The pH of this soln is 4.6–4.8.

(b) *Alkaline ferricyanide soln.*—16.5 g of pure dry  $K_3Fe(CN)_6$  and 22 g of anhydrous  $Na_2CO_3$  in 1 liter of  $H_2O$ . The  $K_3Fe(CN)_6$  soln is 0.05 *N*. It maintains its strength for a long period of time if kept in a dark glass bottle away from the light. (The best C.P. grade of this salt purchased on the market may ordinarily be depended upon to be free from moisture and impurities.)

(c) *Sodium thiosulfate soln.*—0.05 *N*. 12.41 g of  $Na_2S_2O_3 \cdot 5H_2O$  per liter. Select only the clear crystals from the best C.P. grade. If redistilled  $CO_2$ -free  $H_2O$  (the second distillation being made after the addition of a small quantity of alkaline K permanganate to the first distillation, to destroy all traces of organic matter) is used in making up this soln, it will retain its normality for a long time, whereas with ordinary distilled  $H_2O$  it is likely to deteriorate slowly on standing. Check the ferricyanide against the thiosulfate soln as follows: To 10 cc of the ferricyanide soln add 25 cc of the acetic acid reagent (d) followed by 1 cc of 50% KI and 2 cc of soluble starch soln. Titrate with the Na thiosulfate soln. (It should require exactly 10 cc of the Na thiosulfate to completely discharge the blue starch-iodine color.) Standardize the Na thiosulfate soln against pure I soln if necessary.

(d) *Acetic acid soln.*—200 cc of glacial acetic acid, 70 g of KCl, and 20 g of  $ZnSO_4 \cdot 7H_2O$  per liter.

(e) *Potassium iodide soln.*—50% soln of KI. Add 1 drop of NaOH for each 100 cc of soln to prevent or substantially delay deterioration of the soln (with liberation of I) on standing, which will otherwise occur. The soln must be colorless.

(f) *Soluble starch soln.*—1% of soluble starch in 30% NaCl soln. Prepare soluble starch suspension and pour slowly into boiling  $H_2O$ . Add salt and make to volume. The soln should be transparent and colorless.

## 44

## PROCEDURE

(Total maltose after diastasis for 1 hour.)

Introduce 5 g of flour and a teaspoonful of ignited quartz sand into a 100 or 125 cc Erlenmeyer flask, and mix by rotating the flask. Add 46 cc of buffer soln, and again mix by rotating the flask until all the flour is in suspension. (The flask and all ingredients should be *individually* brought to 30° before being mixed together.) Digest for 1 hour at 30°, preferably in an accurately controlled water thermostat, shaking the flask (by rotation) every 15 min. At the end of the hour add 2 cc of  $H_2SO_4$  ( $3.58 \pm 0.05$  *N*, approximately 1+9), and mix thoroly. Add 2 cc of 12% sodium tungstate soln, mix, and let stand a min. or two. Filter thru paper (No. 4 Whatman or its equivalent), discarding the first 8 or 10 drops, and pipet 5 cc of the filtered extract into a test tube of approximately 50 cc capacity (18–20 mm diameter). Pipet exactly 10 cc of the ferricyanide soln into the 5 cc of extract in the test tube, and immerse the test tube in a vigorously boiling water bath; the surface of the liquid in the test tube should be 3–4 cm below the surface of the boiling  $H_2O$ . (The delay between the filtering of the extract and the treatment in the boiling water bath should not be more than 15–20 min. Further delay may cause a slight error due to sucrose hydrolysis in the acid soln.) Allow the test tube to remain in the boiling water bath for *exactly* 20 min. Cool the test tube and its contents under running  $H_2O$ , and pour at once into a 100 or 125 cc Erlenmeyer flask. Rinse out the test tube with 25 cc of the acetic acid soln, and add to the contents of the Erlenmeyer flask, with thoro mixing. Then add 1 cc of the KI soln followed by 2 cc of the starch soln, and mix thoroly. Titrate with 0.05 *N* Na thiosulfate to the complete disappearance of the blue color (a 10 cc buret is recommended). Subtract the



number of cc of 0.05 *N* Na thiosulfate used in the titration from 10, which gives cc of 0.05 *N* ferricyanide reduced to ferrocyanide by the reducing sugars in the flour extract. This value represents a definite quantity of maltose, which may be ascertained by consulting the table (45). When 5 cc of flour extract is used, as herein specified, it is necessary merely to multiply the mg of maltose by 20 to obtain mg of maltose per 10 g of flour in 1 hour's diastasis. This is the value that is recorded and reported as the measure of the diastatic value of the flour in question.

The foregoing specifications may be used with all ordinary flours whose values for mg of maltose produced by 10 g of flour in 1 hour will seldom, if ever, exceed 350. For material giving higher values, such as products from malted or sprouted grain, use smaller portions of extract, *i.e.*, 1, 2, or 3 cc instead of 5 cc. In such cases, however, add enough distilled H<sub>2</sub>O to make up the difference, and use a different factor for converting results into mg of maltose per 10 g of flour. Thus, when 2 cc of extract is used, multiply the value obtained from the table by 50 instead of 20. If the material in the test tubes is colorless instead of yellow, after treatment in the boiling water bath, and gives no blue color upon the addition of KI, it is apparent that there was more than enough maltose to reduce all the ferricyanide, and that the determination must be repeated with a smaller quantity of extract.

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Maltose conversion table\*

0.05 <i>N</i> FERRI- CYANIDE REDUCED	MALTOSE EQUIVALENT	0.05 <i>N</i> FERRI- CYANIDE REDUCED	MALTOSE EQUIVALENT	0.05 <i>N</i> FERRI- CYANIDE REDUCED	MALTOSE EQUIVALENT	0.05 <i>N</i> FERRI- CYANIDE REDUCED	MALTOSE EQUIVALENT
cc	mg	cc	mg	cc	mg	cc	mg
0.1	0.2	2.6	4.2	5.1	8.3	7.6	12.3
0.2	0.3	2.7	4.4	5.2	8.4	7.7	12.5
0.3	0.5	2.8	4.5	5.3	8.6	7.8	12.7
0.4	0.6	2.9	4.7	5.4	8.7	7.9	12.9
0.5	0.8	3.0	4.9	5.5	8.9	8.0	13.0
0.6	1.0	3.1	5.0	5.6	9.1	8.1	13.2
0.7	1.1	3.2	5.2	5.7	9.2	8.2	13.4
0.8	1.3	3.3	5.3	5.8	9.4	8.3	13.5
0.9	1.5	3.4	5.5	5.9	9.6	8.4	13.7
1.0	1.6	3.5	5.7	6.0	9.7	8.5	13.9
1.1	1.8	3.6	5.8	6.1	9.9	8.6	14.0
1.2	1.9	3.7	6.0	6.2	10.0	8.7	14.2
1.3	2.1	3.8	6.2	6.3	10.2	8.8	14.4
1.4	2.3	3.9	6.3	6.4	10.4	8.9	14.6
1.5	2.4	4.0	6.5	6.5	10.5	9.0	14.8
1.6	2.6	4.1	6.6	6.6	10.7	9.1	15.0
1.7	2.8	4.2	6.8	6.7	10.9	9.2	15.2
1.8	2.9	4.3	7.0	6.8	11.0	9.3	15.4
1.9	3.1	4.4	7.1	6.9	11.2	9.4	15.6
2.0	3.2	4.5	7.3	7.0	11.3	9.5	15.9
2.1	3.4	4.6	7.5	7.1	11.5	9.6	16.1
2.2	3.6	4.7	7.6	7.2	11.7	9.7	16.5
2.3	3.7	4.8	7.8	7.3	11.8	9.8	17.0
2.4	3.9	4.9	7.9	7.4	12.0	9.9	-
2.5	4.1	5.0	8.1	7.5	12.2	10.0	-

\* Prepared by applying the specified procedure to standard solutions of pure maltose and using all reagents in the quantities and volumes precisely as used for flour extracts.

46

BLANK DETERMINATION

A blank determination, designed to indicate the quantity of reducing sugar originally present in the flour—the value for which presumably should be deducted from the total maltose value after 1 hour's diastasis—has been generally regarded as an

essential step in the estimation of flour diastatic activity. This operation, however, is ordinarily unnecessary when dealing with flour milled from *sound* wheat, because the quantity of reducing sugars originally present as such is so small and so nearly constant that it may be disregarded for all practical purposes. The blank determination may therefore be conveniently omitted in ordinary routine testing. It need be used only when there is occasion to doubt the soundness of the wheat, or in cases where there is known to have been an appreciable quantity of frosted, sprouted, heat-damaged, or otherwise unsound kernels in the wheat from which the flour was milled.

To make the blank determination, proceed as follows: Add to 5 g of flour and a teaspoonful of quartz sand in a 100 or 125 cc Erlenmeyer flask 48 cc of 0.4% (by volume)  $H_2SO_4$  (preferably pre-cooled to ice-water temp.). Shake the mixture thoroly again, allow to stand 2 min. and filter thru a No. 4 Whatman (or its equivalent) paper. Using 5 cc of the clear filtrate, proceed according to 44.

#### VISCOSITY OF ACIDULATED FLOUR<sup>24</sup>

47

##### APPARATUS

*MacMichael viscosimeter*.—Standardize with a 60% sucrose soln at different temps., using a No. 30 wire disk bob, speed 12 r.p.m. Before making any determinations check for zero point, correctly level the machine, check speed, and see that torsion wire is adjusted flush with chuck in end of spindle.

48

##### PREPARATION OF FLOUR SUSPENSION

Use a constant weight of protein. Place 50 cc of distilled  $H_2O$  in a mortar (50 cc automatic pipet is convenient for measuring the  $H_2O$  required), add flour, and work into a smooth paste (about 2.5 min.). Use as little pressure on pestle as possible to avoid the formation of foam and development of gluten. Pour the flour paste into the viscosimeter cup and rinse out mortar with a second 50 cc of distilled  $H_2O$ . Pour the rinsings into the cup, add 2 drops of caprylic alcohol, stir a few times with a disk bob, place the cup on the machine, and stir a few times. The sample is now ready for the first viscosity reading. Digest for 60 min.

49

##### DETERMINATION

Correctly hang the bob in the freshly agitated suspension and take the reading. Dampen the swings by placing a finger on the indicator pointer and then gently touching the swinging dial.

Make the second and subsequent readings after adding 1, 2, 2, and 2 cc of normal lactic acid, respectively, a total of 7 cc. Obtain the second reading as follows: After stopping the motor, detach the bob with the right hand and use for agitating the suspension, using an up and down, backward and forward, circular motion. With the left hand add 1 cc of acid (use a 1 cc pipet). Stir with the bob 25 times after commencing flow of the acid (11 seconds). Resuspend the bob and take reading. Obtain the remaining readings as directed above except to add 2 cc of acid each time from a 2 cc pipet.

#### BAKED CEREAL PRODUCTS

##### BREAD

50

##### PREPARATION OF SAMPLE<sup>25</sup>—TENTATIVE

(To be used when total solids of original entire loaf is not desired.)

Cut the loaf, or  $\frac{1}{2}$  the loaf, of bread into slices 2–3 mm thick. Spread the slices on paper and allow them to dry in a warm room until sufficiently crisp and brittle

to grind well in a mill. Grind the entire sample to pass a 20-mesh sieve, mix well, and keep in an air-tight container.

#### 51 TOTAL SOLIDS IN AN ENTIRE LOAF OF BREAD<sup>25</sup>—OFFICIAL

Accurately weigh the loaf of bread immediately upon receipt (*A*), using scales sensitive to at least 0.2 g. (When determining whether bread is in conformity with the Department of Agriculture standards do not weigh the loaf sooner than 1 hour after removal from the oven.) Should accurate weighing be impossible at this time, seal the sample in an air-tight container and accurately weigh as soon thereafter as is practicable (*A*). Preserve the sample in such a manner that no loss of bread solids can occur, whereby the loss would be calculated as moisture. Cut the bread into slices 2–3 mm thick ( $\frac{1}{2}$  of the loaf may be used). Spread the slices on paper; allow them to dry in a warm room (approximately 15–20 hours); and when apparently dry, break into fragments. If the bread is not entirely crisp and brittle, allow it to dry longer—until it is in equilibrium with the moisture of the air—in order that no moisture changes may occur during grinding. Quantitatively transfer the air-dried bread to the scale pan and accurately weigh (*B*). Grind the sample just to pass a 20-mesh sieve, mix well, and keep in an air-tight container. Determine the percentage of total solids (*C*) of the ground sample as directed under 3 or 5. Calculate total solids of the bread from the formula:

$$T.S. = \frac{\frac{B \times C}{100} \times 100}{A}, \text{ or } \frac{B \times C}{A}, \text{ in which}$$

*A* = weight of loaf (or  $\frac{1}{2}$  loaf) at time of receipt

*B* = weight of the air-dried sliced bread; and

*C* = percentage of total solids in the prepared ground sample.

#### TOTAL SOLIDS OF AIR-DRIED GROUND SAMPLE<sup>25</sup>

##### 52 *Method I—Official*

Use 2 g of the prepared sample, 51, and proceed as directed under 3.

##### 53 *Method II—Official*

Proceed as directed under 4.

#### MILK SOLIDS IN BREAD<sup>26</sup>

##### 54 *Fat Method*

Slice one loaf of bread, place in a wire rack, and allow to dry overnight, or until it is sufficiently dry to grind. Grind the bread to approximately the size of the openings in a 20-mesh sieve, mix, sample and transfer 50 g to a 600 cc beaker. Add 100 cc of H<sub>2</sub>O and mix. Add 100 cc of HCl, mix, cover, and place on the steam bath for one hour, stirring well 6 or 7 times. Cool in a cold (15° or less) water bath, add 50 cc of ice cold H<sub>2</sub>O, and stir. Add 10 g of filter cel, stir, and mix in completely. Prepare a 10 mm Büchner funnel as follows:

Place two No. 590 S & S 9 cm filter papers in the funnel and apply suction. Mix 10 g of filter cel with 50 cc of H<sub>2</sub>O and rapidly pour mixture into funnel. This should make a smooth even layer of the filter cel over the whole filter paper, with no crack or opening. Filter the sample immediately. Rinse out the beaker several times with

ice cold  $H_2O$ . Just before the filtration is complete, wash down the sides of the Büchner with about 100 cc of ice cold  $H_2O$ . Up to this point do not allow the pad to suck dry. Continue with the suction until the filter cel pad seems dry. Transfer this mass, without filter paper, from the Büchner to the original beaker. Rinse off the filter paper and funnel with petroleum ether and add the ether to the beaker containing the dry mass. Break up the mass with a rod, and dry on the steam bath to remove the water. Heat in the oven at  $100^\circ$  only until dry (about 1 hour). Add 25 g of anhydrous sodium sulfate and break up any lumps. Prepare a large Knorr extraction tube of about 200 cc capacity (glass tubing 5 cm in diameter with a height of 12 cm from shoulder to the top of the tube). Pack the tube with asbestos tamped tightly to form a pad about  $\frac{3}{8}$  in. thick. Insert the stem of the tube into a two-holed rubber stopper in a filtering bell jar connected to a suction thru a two-way stopcock. Place a 500 cc Erlenmeyer flask within the bell jar so that the stem of the tube passes thru the neck of the flask. To the cool beaker and contents add 150 cc of mixed ethyl and petroleum ethers, equal parts of each, and macerate against the sides of the beaker with a medium sized stiff metal spatula for 3 or 4 min. Decant onto the extraction tube. Add to the beaker 80 cc of the mixed ether. Work as before for 2 min., likewise decant. Transfer the contents of the beaker to the extraction tube, suck dry, and tamp with a flattened stirring rod until all ether is removed. To the material in the tube add 100 cc of the mixed ethers that have just previously been used to rinse out the beaker, mix thoroly with a stirring rod a few minutes, allow to stand a minute, then suck dry, and tamp the material as before. Likewise make two additional extractions, turning suction on and off carefully to avoid loss of sample in the Erlenmeyer flask. Hang a thread in the flask from the top so that it touches the bottom. (Time may be saved by transferring to a tall-form 1 liter beaker.) Evaporate on the steam bath, completely transfer the fat with small amounts of petroleum ether to a tared 150 cc beaker, carefully evaporate the ether on the steam bath, dry at  $100^\circ$  to constant weight (about 30 min.), cool, and weigh. Figure the percentage of total fat on moisture-free basis.

Weigh duplicate samples of 1 g (within  $\pm .03$  g) of fat into a 300 cc Florence flask, add 1 cc of NaOH (1+1), stopper, and place on the steam bath 1 hour. Cool, add a few pieces of pumice stone previously ignited, 138 cc of distilled  $H_2O$ , and 3 cc of  $H_2SO_4$  (1+1), and proceed as directed in XXXI, 27, using the same apparatus. Use 0.02 *N* NaOH for titration and report number of cc per 1 g of fat. Multiply the cc of 0.02 *N* NaOH used by 1.1 and divide by the weight of fat taken to obtain the "fat number." Calculate butter fat and milk solids from the "fat number" and total fat as follows:

- Let  $A$  = % total fat on moisture-free basis,  
 $B$  = "fat number" as determined by titration,  
 $C$  = % butter fat in bread on moisture-free basis,  
 31.5 = "fat number" on butter fat,  
 1.0 = "fat number" on fat from water bread,  
 7.7% = milk solids on moisture-free basis in milk bread  
 2.3% = milk fat on moisture-free basis in milk bread,  
 $D$  = % milk solids in bread on moisture-free basis

$$\text{Then } C = \frac{A(B-1.0)}{31.5} \quad D = \frac{7.7 \times A(B-1.0)}{2.3 \times 31.5}.$$

55

*Citric Acid Method*

To a weight of air-dried bread equivalent to 77.7 g of moisture-free bread in a 500 cc volumetric flask, add 400 cc of a mixture containing 25 cc  $N H_2SO_4$ , 20 cc of a 20% phosphotungstic acid soln, 55 cc of  $H_2O$ , and sufficient 95% alcohol to make 500 cc. Shake for 5 min., make to mark, and allow to stand overnight. Re-adjust to mark with 95% alcohol, shake 5 min., and filter with suction on paper in a 12 cm Büchner funnel. Transfer 325 cc of the filtrate to a centrifuge bottle, add 30 cc of Pb acetate soln (75 g of the salt plus 1 cc of glacial acetic acid diluted to 250 cc with  $H_2O$ ), shake 5 min., and centrifuge at about 900 r.p.m. for 15 min. Decant the supernatant liquid (disregard turbidity); allow to drain, transfer the residue with about 150 cc of  $H_2O$  to a 250 cc volumetric flask, and thoroly saturate with hydrogen sulfide. Make to mark with  $H_2O$ , shake thoroly, and filter thru a large folded filter. Evaporate 200 cc of the clear filtrate in a 500 cc Erlenmeyer flask over a free flame to about 75 cc. Cool to 45–50°; add 10 cc of  $H_2SO_4$  (1+1), 5 cc of KBr soln (15 g in 40 cc of  $H_2O$ ), and 15 cc of permanganate soln (5 g of  $KMnO_4$  diluted to 100 cc). After about 2 min., stopper the Erlenmeyer, shake vigorously, and allow to stand 3 min. longer. Add 20 cc of ferrous sulfate soln (40 g of the salt plus 1 cc of  $H_2SO_4$  diluted to 100 cc with  $H_2O$ ), cool to about 15°, and shake vigorously until the pentabromacetone has crystallized (lace-like deposit on the walls of the flask). Place in a refrigerator at about 15° overnight. Avoid a temp. of less than 15° since at lower temp. there is a tendency for the pentabromacetone to freeze on the sides of the flask. Filter, and dry the pentabromacetone as directed under **XXVI, 31**. If the drying is done by aspiration, 20 min. should be sufficient time. It is also advisable to cool the air current by placing the vessel containing the  $H_2SO_4$  into ice cold  $H_2O$ . Weight of pentabromacetone in g  $-0.004 g \times 75 =$  per cent whole milk solids in moisture-free bread.

56

ASH—OFFICIAL<sup>27</sup>

Use 3–5 g of the prepared sample, 50, and proceed as directed under 5.

57

## CHLORIDES IN ASH—OFFICIAL, FIRST ACTION

Proceed as directed under 72.

58

## PROTEIN—OFFICIAL

(Organic and Ammoniacal Nitrogen.)

Determine N as directed under II, 21, 23, or 25, using 2 g of the prepared air-dried ground sample, 50. Multiply the percentage of N by the factor 5.7 to obtain the percentage of protein.

59

## FAT (ACID HYDROLYSIS METHOD)—OFFICIAL

Proceed as directed under 11.

60

## CRUDE FIBER—OFFICIAL

(For bread and other baked products not containing fruit.)

Proceed as directed under **XXVII, 27**.

EXPERIMENTAL BAKING TEST<sup>28</sup>—TENTATIVE

61

## EQUIPMENT

(1) *Mixer*.—Hobart-Swanson.

(2) *Fermentation bowls*.—Graniteware "oatmeal bowls." Top diameter 14.5 cm, bottom diameter 5 cm, and depth 6.5 cm.

(3) *Fermentation cabinet*.—Should have accurate temp. control ( $\pm 0.5^\circ$ ) and maintain a relative humidity of at least 75%.<sup>28</sup>

(4) *Baking pans*.—Tall or low form, made of tin, with the following inside dimensions:

LOW FORM TINS				TALL FORM TINS			
		cm	cm		cm	cm	
Length	top	11.5	bottom 9.5	top	10.5	bottom 9.3	
Width	top	7.0	bottom 5.5	top	6.0	bottom 5.3	
Depth		5.0		ends	6.8		
				sides	8.5		

NOTE: Investigation has shown that the low form tins give significantly higher volumes, lower variability between replicates, and more uniform crumb grain and texture than do the tall form tins; they also correspond more closely to commercial pans. The low form tins are especially recommended for research work. In reporting results of baking tests the type of pan used should be specified.

(5) *Baking oven*.—Should maintain a temp. of  $230^\circ\text{C}$  ( $\pm 5^\circ$ ) and preferably be equipped with a rotating shelf.

(6) *Thermometers*.—

(a) *Fermentation cabinet and dough testing*.—A.A.C.C. official thermometer graduated from  $15^\circ$  to  $40^\circ\text{C}$  or equivalent Fahrenheit range.<sup>29</sup>

(b) *Oven*.—A.A.C.C. official thermometer graduated from  $100^\circ$  to  $260^\circ\text{C}$  or equivalent Fahrenheit range.

(7) *Volume measuring apparatus*.<sup>30</sup>—Should be accurately calibrated. A set of aluminum loaf models is convenient. It is suggested that four standards of approximately 300, 400, 500, and 600 cc be used. Plot these against the loaf volume readings of the measuring device to obtain the calibration curve of the apparatus.

## 62 PREPARATION OF YEAST SUSPENSION AND SALT-SUGAR SOLUTION<sup>31</sup>

As the absorption of flours exceeds 50% it is convenient to prepare a stock soln of sugar and salt and a yeast suspension of such a strength that 25 cc of each contains the required quantities of these ingredients per loaf. The volume displacement of 3 g of fresh compressed yeast is 2.5 cc and that of 1 g of salt and 2.5 g of sugar when dissolved together to make a total volume of 25 cc is 1.86 cc. The quantities of yeast, salt and sugar, and  $\text{H}_2\text{O}$  required to prepare solns for varying numbers of loaves are shown below:

Table for preparing yeast suspension and salt-sugar solution required for specified numbers of loaves using 25 cc each per loaf

NUMBER OF LOAVES	YEAST SUSPENSION		SALT-SUGAR SOLUTION		
	YEAST	WATER	SUGAR	SALT	WATER
	gram	cc	gram	gram	cc
5	15	112.5	12.5	5.0	115.7
10	30	225.0	25.0	10.0	231.4
15	45	337.5	37.5	15.0	347.1
20	60	450.0	50.0	20.0	462.8
25	75	562.5	62.5	25.0	578.5
30	90	675.0	75.0	30.0	694.2

NOTES: 1. Water added per loaf in form of stock solns:

	cc
(a) 25 cc of yeast suspension contains	22.5
(b) 25 cc of salt-sugar soln contains	23.1
Allowance in computing absorption	45.6

2. Agitate the yeast suspension before and during the removal of an aliquot.

3. Keep the soln at a temp. such that when mixed with the flour and any extra H<sub>2</sub>O required, the doughs will come from the mixer at 30° (±0.5°).

63

## BASIC FORMULA

*Flour*.—100.0 g (±0.1 g) on 15% moisture basis (85.0 g dry matter). Determine moisture by 130° air oven or vacuum oven method (2 and 4).

*Yeast*.—3.0 g (3%) fresh compressed yeast.

*Salt*.—1.0 g (1%) 99.5% pure.

*Sugar (sucrose)*.—2.5 g (2.5%).

*Water*.—Sufficient to yield dough of standard consistency (not too "tight" or too "slack").

64

## STANDARD PROCEDURE

(1) *Mixing*:

(a) Place the flour in the bowl of the mixer, add 25 cc each of the yeast suspension and salt-sugar soln plus sufficient additional H<sub>2</sub>O to bring the dough to the desired consistency. If this is not accomplished before the dough has formed, discard the mix and repeat the test. Mix for 1 min. The doughs should come from the mixer at a temp. of 30° (±0.5°). If 100 g of flour yields too small a dough for thoro mixing, use a larger quantity and scale the dough to proper weight after mixing.

(b) *Alternative procedures*:

If an official mixer is not available, use any method of mixing that will thoroly incorporate the ingredients and produce a smooth dough with a minimum development of gluten. A Hobart mixer equipped with 2 dough arms or a cake paddle may be substituted. Even hand mixing may be resorted to when a machine mixer is not available.<sup>32</sup>

(2) *Calculation of absorption*.—% absorption (15% moisture basis) =  $45.6 + W - (100 - F)$ , where W = cc of distilled H<sub>2</sub>O added and F = the weight of flour.

(3) *Fermentation*:

	Minutes
First punch after	105
Second punch after additional	50
Mold after additional	25
Total	180

Remove the dough from the mixing bowl, fold 20 times in the hands, put in the fermentation bowl, and place in the fermentation cabinet. After 105 min. remove the dough from the bowl and fold 15 times in the hands; round up and return dough to bowl and cabinet. Give second punch after an additional 50 minutes' fermentation, folding the dough 10 times and rounding up as before. After further fermentation of 25 min., mold the dough and pan as directed.

(4) *Molding and panning*.—Place the dough on a piece of cotton or canvas belt-ing. Press with the heel of the hand until the dough is uniformly flat and circular

(1). Loosen the dough from the belting and turn on reverse side. Fold over two opposite sides so that they overlap to a considerable degree (2). Turn the dough over and again flatten with heel of hand. Holding one end, again loosen from molding surface and turn on reverse side with the seam of the dough running from the operator. Starting at the more remote end, roll toward the operator, folding as tightly as possible (3). Seal the seam tightly and with the seam on the bottom, seal the ends by punching them vertically. Roll the dough lightly under the palm of the hand, adjusting it to the length of the pan and place in pan with the seam down. (The length of the dough should not exceed that of the pan prior to the final light



FIG. 21.—PREPARING THE DOUGH FOR PANNING

rolling.) *Use no dusting flour in the molding process.* Grease the pans very lightly and only when absolutely necessary to prevent the loaves from sticking to the sides of the pans.

(5) *Proofing.*—Proof 55 min. under the same conditions used for fermentation.

(6) *Baking.*—Bake 25 min. at a temp. of  $250^{\circ} (\pm 5^{\circ})$  with the thermometer placed at the level of the top of the baking pans at a distance of 5 cm on the side next the axis of rotation. (Precise control of temp. is essential.) Place an open pan of  $H_2O$  in the baking oven.

(7) *Measurement.*—Weigh the loaf and measure its volume 30 min. after removal from the oven. Place the loaves in a fairly air-tight cabinet until the following morning.

(8) *Scoring.*—Score the loaves the day following baking for the external characteristics, crust color and symmetry, and for the internal characteristics, crumb color, grain, and texture.<sup>33</sup>



NOTES: 1. The basic formula and standard baking procedure outlined are designed primarily for hard wheat flours to serve as a point of reference and a basis for any supplementary tests that may be considered appropriate. Any additional testing procedure designed to reveal particular characteristics of flours may be used but only one variable at a time should be introduced. Such tests as mechanical modification by varying the mixing time, the addition of diastatic supplements, oxidizing agents as potassium bromate, and the use of varying periods of fermentation have been found particularly valuable.

2. Fresh baker's yeast from the same source should be used in each investigation, and the supply should be stored in the ice box in containers to prevent evaporation of moisture. The outside portions of the yeast cake should be removed before being used. Studies on the effect of aging yeast on loaf volume are somewhat contradictory and it is advisable to secure a fresh supply every 2 days.<sup>34</sup>

3. It is advisable to prepare five "dummy" doughs at the beginning and end of each regular day's baking in order to provide more uniform oven conditions at the beginning and end of the series.

65

## SUPPLEMENTARY TESTS

(1) *Fermentation*.—Basic procedure varying fermentation time only.

(2) *Addition of  $KBrO_3$* .—Basic procedure with addition of  $KBrO_3$  in increments of 1 mg per loaf.

(3) *Sugar variation*.—Baking with increments of 2.5 g recommended for varying amounts of sugar in formula.

(4) *Mechanical modification*.—Variation of mixing time.

BAKED PRODUCTS OTHER THAN BREAD<sup>34</sup>

66

## SOLIDS

Proceed as directed under 50 and 51.

67

CRUDE FIBER<sup>35</sup>

Proceed as directed in XXVII, 27.

## MACARONI PRODUCTS

68

COLLECTION AND PREPARATION OF SAMPLE<sup>35</sup>—TENTATIVE

Select from the lot to be analyzed sufficient strips or pieces to assure a representative sample. Break these into small fragments with the hands or in a mill and mix well. Grind 300–500 g in a mill until all the material just passes thru a 20-mesh sieve. Keep the ground sample in a sealed container to prevent moisture changes.

## TOTAL SOLIDS AND MOISTURE

69

*Vacuum Oven Method<sup>35</sup>—Official, First Action*

Determine the total solids in the prepared sample as directed under 3.

70

*Air Oven Method—Official*

Proceed as directed under 4, using a sample prepared as directed under 68.

71

## ASH—OFFICIAL

Proceed as directed under 5, using 3–5 g of the prepared sample, 68.

72

## CHLORIDES IN ASH AS SODIUM CHLORIDE—OFFICIAL

Dissolve the ash obtained under 71 in  $HNO_3$  (1+9), filter, wash the filter paper with hot  $H_2O$ , and determine Cl in the combined filtrate and washings as directed

under **XII, 35** or **37**. Calculate the Cl to its equivalent of NaCl. (This NaCl value deducted from the total ash does not give NaCl-free ash.)

73

**FAT (ACID HYDROLYSIS METHOD)<sup>17</sup>—OFFICIAL**

Place 2 g of the sample in a Röhrig or Mojonnier fat extraction tube, add 2 cc of 95% (by volume) alcohol, to prevent lumping on addition of the acid, and shake so as to moisten all particles. Add 10 cc of HCl (25+11), mix well, set the tube in a water bath held at 70–80°, and shake at frequent intervals for 30–40 min. Fill to within 1–2 cc of the mark with 95% alcohol and cool. Add 25 cc of ethyl ether and shake the mixture well. Then add 25 cc of redistilled petroleum ether (b. p. below 60°) and mix well. Let stand until the upper liquid is practically clear and proceed as directed under **11**, beginning "Draw off as much as possible."

74

**CRUDE FIBER—OFFICIAL**

Proceed as directed under **XXVII, 27**.

75

**PROTEIN<sup>18</sup>—OFFICIAL**

Determine the N as directed under **II, 21, 23**, or **25**, using 1 g of the prepared sample, **68**. Multiply the percentage of N by the factor 5.7 to obtain the percentage of protein.

**76 WATER-SOLUBLE PROTEIN-NITROGEN PRECIPITABLE BY 40 PER CENT ALCOHOL—OFFICIAL, FIRST ACTION**

Proceed as directed under **26**.

**77 LIPOID AND LIPOID PHOSPHORIC ACID (P<sub>2</sub>O<sub>5</sub>)—OFFICIAL, FIRST ACTION**

Proceed as directed under **27** and **28**.

**UNSAPONIFIABLE RESIDUE—TENTATIVE**

78

*Modified Kerr-Sorber Method*

Proceed as directed under **29**.

**79 EXTRACTION, SEPARATION, AND IDENTIFICATION OF COLORING MATTER IN MACARONI, EGG NOODLES, AND SIMILAR PRODUCTS<sup>19</sup>—OFFICIAL, FIRST ACTION**

Place approximately 500 g of the coarsely ground sample (depending upon the quantity of color present) in a liter Erlenmeyer flask, add about 700 cc of 80% alcohol, and shake at intervals for 24 hours or until the color is imparted to the solvent. Place the flask with contents in the refrigerator overnight to permit dissolved protein matter to precipitate out. Filter, and evaporate the filtrate to 100 cc. Add to the filtrate about  $\frac{1}{2}$  volume of 25% salt soln and a slight excess of ammonia; cool, and transfer to a separatory funnel. Extract this mixture with an equal volume of ligroin or petroleum ether; separate the lower layer and repeat extractions with additional portions of the solvent, until no more color is extracted. Reserve the lower layer, if colored, for further treatment; if colorless, discard. Combine the petroleum ether extracts and wash with several small portions of ammonia water (1+50) to remove any material mechanically adhering to the solvent. This ethereal soln will contain the fats, and also may contain the oil-soluble coal tar dyes, which may be identified by the procedure under (1). If colored, immediately acidify the alkaline aqueous soln freed from fat and oil-soluble coal tar dyes with acetic acid

and extract in 25 cc portions with two 50 cc volumes of ether. The solvent, if colored, may contain turmeric, annatto, and a trace of saffron. For their identification use procedure (2). If the original aqueous soln freed from ether-soluble colors should still be colored, water-soluble dyes may be suspected, in which case the following procedure is recommended: Extract the aqueous soln with 50 cc portions of amyl alcohol to remove the balance of the saffron, as well as the common orange dyes (S & J numbers 85, 86, 13) and martius yellow. For their separation proceed as directed under **XXI**. Draw off the lower aqueous layer, which, if colored, may contain naphthol yellow S, tartrazine and sunset yellow. Extract these dyes with amyl alcohol after acidifying the soln with HCl to make approximately 1 *N*. Remove tartrazine from the solvent with 0.25 *N* HCl. Sunset yellow will also be removed at this stage with a slightly lower acid concentration, and naphthol yellow S from a nearly neutral soln. Confirm with wet and spot reactions. The extracted solns are usually very dilute, therefore it is advisable to concentrate by evaporation over a steam bath, and if not clear, to add about 5 cc of 25% salt soln to break up slight emulsions by precipitating the protein matter, filter, and test the filtrate by dyeing and coupling. This coupling test is carried out as follows: Treat about 10 cc of the filtered soln with excess of bromine, destroy the excess with a saturated soln of hydrazine sulfate, and immediately pour into a sodium carbonate soln of alpha naphthol. In the presence of tartrazine or sunset yellow a pink color will be produced. It is advisable to run a blank determination on the above test for comparison.

(1) Extract the original petroleum ether extract with two or three 10 cc portions of a mixture consisting of 1 part of HCl and 5 parts of acetic acid.

In the presence of S & J numbers 7 or 16, yellow OB or yellow AB, a pink or red color is obtained. Test a small portion of this acid extract with a few drops of  $\text{SnCl}_2$ , which in the presence of the above dyes will cause either decolorization or a decided fading. Dilute the balance of the acid extract with  $\text{H}_2\text{O}$ , make slightly alkaline, and extract the color with petroleum ether. Wash the solvent with 2-5 cc portions of  $\text{H}_2\text{O}$  to remove excess of alkali. Test an approximately 5 cc portion of the petroleum ether extract with formaldehyde and acetic anhydride as directed under **XXI**, 9(a). Evaporate another 5 cc portion of the petroleum ether extract to dryness in a small evaporating dish and observe spot tests with HCl and  $\text{H}_2\text{SO}_4$ . Evaporate to dryness the balance of the petroleum ether extract in a small casserole and dissolve the residue in dilute alcohol. Dye some silk strands, preferably using a slightly alkaline soln. Compare the spot tests obtained with Table 10, **XXXI**. If they do not agree with the tables, a mixture of dyes may be present, which will necessitate a separation according to the pH concentration.

The remaining coloring matters in the ligroin extract may be due to the natural coloring matter of wheat, or to the coloring matter of egg. The coloring principle of egg yolk, lutein, when heated with alcoholic ferric chloride, will produce a green coloration. However, this test is not specific for lutein, as carotin and xanthophyll give similar reactions.

(2) Wash the ether extract with 5 cc portions of  $\text{H}_2\text{O}$  to remove excess of acid. To remove annatto and the traces of saffron, wash successively with 20 cc portions of 5%  $\text{NaHCO}_3$  soln. Divide this alkaline soln into two portions. Heat one portion to 60° on the steam bath and dye the color on unmordanted cotton, and compare spot tests with a standard. Acidify the remaining portion of the alkaline annatto soln with acetic acid and re-extract with ether. Divide the ethereal extract into two small casseroles and evaporate to dryness. Dissolve the contents of one casserole

in 10 cc of ammonia water (1+9) and impregnate it on a strip of cotton or filter paper. An orange yellow to an orange red coloration is obtained depending upon the amount of dye present. Dry the filter paper or cotton, add a drop of 40%  $\text{SnCl}_2$ , and again dry. In the presence of annatto a purple stain is produced. Spot the contents of the other casserole with  $\text{H}_2\text{SO}_4$  and  $\text{HNO}_3$ , when a blue and a greenish blue color are obtained. Transfer two portions (of about 10 cc each) of the original ether extract from which annatto has been removed, into test tubes and treat with an equal volume of 10%  $\text{NaOH}$  and an equal volume of  $\text{HCl}$  (1+1), respectively. In presence of turmeric (curcuma) the alkaline soln will be reddish brown, while the acid soln will be red. Turmeric can further be confirmed by its behavior with boric acid. Apply this test as follows: Shake a portion of the original ether extract with an equal volume of 70% alcohol and to this add 1/10 volume of  $\text{HCl}$ , mix, and divide the soln equally into two test tubes. To one tube add a few crystals of boric acid and shake. Use the other tube as a control. In the presence of turmeric, a red color will be produced after a short time.

(3) To separate and identify saffron and the orange coal tar dyes, dilute the amyl alcohol extract with two volumes of petroleum ether and extract the mixed dyes with several 10 cc portions of  $\text{H}_2\text{O}$ . To a small portion of this aqueous extract add 1/10 volume of glacial acetic acid and add a few mg of dry sodium hyposulfite to reduce all the azo dyes. This treatment will not affect the saffron, which can then be re-extracted by amyl alcohol. After washing the solvent repeatedly with small portions of  $\text{H}_2\text{O}$  (to remove decomposition products) evaporate to dryness, and confirm the presence of saffron by spot tests. The remainder of the color soln after addition of salt and acetic acid is re-extracted with amyl alcohol and later fractionated from the solvent for S & J numbers 85, 86, 13, by 5%  $\text{Na}_2\text{CO}_3$  soln. Martius yellow if present will still remain in the amyl alcohol and petroleum ether after the removal of the saffron and oranges. In order to prove its presence, evaporate the solvent to dryness and dissolve the residue with 10 cc ammonia (1+9). Divide into two test tubes. Add carefully to one a few crystals of sodium hyposulfite. The presence of martius yellow will manifest itself by the formation of a pink soln. To check its presence use the other subdivision for dyeing, spotting, etc.

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- <sup>2</sup> Ibid., **8**, 665 (1925); **9**, 39 (1926).
- <sup>3</sup> Ibid., **8**, 665 (1925); **9**, 40 (1926).
- <sup>4</sup> Ibid., **7**, 132 (1923).
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- <sup>6</sup> Cereal Lab. Methods, A.A.C.C., 1935.
- <sup>7</sup> J. Assoc. Official Agr. Chem., **6**, 508 (1922); **9**, 41 (1926).
- <sup>8</sup> Ibid., **10**, 33 (1927); **11**, 37 (1928); **17**, 393 (1934); **18**, 76 (1935).
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- <sup>13</sup> J. Assoc. Official Agr. Chem., **7**, 84 (1923); **12**, 40 (1929); **14**, 500 (1931).
- <sup>14</sup> Ibid., **7**, 91 (1923); **9**, 40 (1926).
- <sup>15</sup> Ibid., **7**, 91 (1923); **9**, 41 (1926).
- <sup>16</sup> Ibid., **9**, 45 (1926); **10**, 35 (1927); **11**, 37 (1928).
- <sup>17</sup> Ibid., **8**, 441 (1925); **10**, 33 (1927).
- <sup>18</sup> Ibid., **10**, 108 (1927); **11**, 37 (1928); **16**, 504 (1933); **17**, 65 (1934).
- <sup>19</sup> Ibid., **6**, 68 (1922); **7**, 130 (1923).
- <sup>20</sup> Ibid., **11**, 134 (1928).
- <sup>21</sup> Ibid., **18**, 493 (1935); **19**, 86 (1936).
- <sup>22</sup> Ibid., **17**, 65 (1934).

- <sup>23</sup> Ibid., 15, 572 (1932); 16, 497 (1933); 17, 397 (1934); 18, 76 (1935); 19, 86 (1936); Cereal Chem., 9, 378 (1932); Am. Inst. Baking Bull. 8 (1932).
- <sup>24</sup> J. Assoc. Official Agr. Chem., 18, 76 (1935); Cereal Chem., 11, 121, 299 (1934); Nebraska Agr. Expt. Sta. Bull. 8 (1916).
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- <sup>26</sup> Ibid., 18, 574 (1935); 19, 86 (1936).
- <sup>27</sup> Ibid., 9, 42 (1926).
- <sup>28</sup> Ibid., 12, 41 (1929); Cereal Lab. Methods, A.A.C.C., 1935, p. 74; Cereal Chem., 7, 307, 341, 346 (1930); 8, 233 (1931).
- <sup>29</sup> Cereal Chem., 5, 158 (1928); 7, 362 (1930).
- <sup>30</sup> Ibid., 7, 307, 346 (1930).
- <sup>31</sup> Ibid., 5, 470 (1928).
- <sup>32</sup> Ibid., 158.
- <sup>33</sup> Ibid., 289; 6, 164, 253 (1929); 10, 545 (1933).
- <sup>34</sup> Ibid., 10, 617 (1933); Canadian J. Res., 6, 614 (1933).
- <sup>35</sup> J. Assoc. Official Agr. Chem., 9, 43, 396 (1926); 17, 65 (1934).
- <sup>36</sup> Ibid., 9, 43, 397 (1926); 17, 65 (1934).
- <sup>37</sup> Ibid., 6, 508 (1923); 11, 38 (1928).
- <sup>38</sup> Ibid., 9, 43 (1926).
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## XXI. COLORING MATTERS IN FOODS—TENTATIVE

(The numbers in parentheses and brackets following the name of a dye represent in the first instance the number of that dye as listed in "A Systematic Survey of the Organic Colouring Matters," founded on the German of Drs. G. Schultz and P. Julius, 1904, by Arthur G. Green, while the second number designates the number as listed in the Society of Dyers and Colourists' "Colour Index," first edition January, 1924.)

### 1

#### PIGMENTS AND LAKES

Separate the insoluble pigments, ultramarine, lampblack, etc., which are most commonly used as facings, by washing the sample with  $H_2O$  and allowing the washings to settle. Identify the particles of coloring matter by microscopical examination and treat the residue or purified coloring matter with chemical reagents.

The pigments occasionally encountered are charcoal or other form of carbon, ultramarine blue (principally aluminum, sulfur), prussian blue (principally iron), talcum (principally silica). Charcoal is indifferent towards the usual chemical reagents and can be burned. Ultramarine blue is stable towards alkalies, but is decomposed by dilute  $HCl$  with the evolution of  $H_2S$ . Prussian blue is unaffected by dilute  $HCl$ , but is decomposed by alkalies. Talcum can be confirmed by the purple coloration obtained by fusing with cobalt nitrate (test for aluminum).

Lakes are products formed by combining organic coloring matters with metallic salts. They can be prepared from animal or vegetable coloring matters or from coal tar dyes. As a rule they are insoluble in  $H_2O$ , but are readily decomposed by acids with the liberation of the coloring matter.

A large proportion of the common pigments other than lakes, such as the yellow, brown and red ochres and umbers, are derivatives of the heavy metals and contain Fe, Mn, etc. Others, such as the green and blue compounds, including certain green chlorophyll derivatives, may contain Cu. These pigments may be identified by the usual tests for the respective metals. The analytical properties of the insoluble coloring matters are described in various standard works, some of which are listed under the selected references, especially Farbstofftabellen by Schultz<sup>1</sup> and Colour Index.

#### SOLUBLE COLORING MATTERS AND THEIR LAKES

##### SEPARATION BY WOOL DYEING PROCEDURE:

### 2

#### Water-Soluble Coal Tar Dyes

(a) *Wines, fruit juices, distilled liquors, flavoring extracts, vinegars, beers, sirups, non-alcoholic beverages, and similar products.*—Dilute 20–200 cc of the sample with 1–3 volumes of  $H_2O$ , neutralize with  $NH_4OH$  (1+9) if necessary, and boil or heat on a steam bath with a small piece of white woolen cloth (nun's veiling). If the mixture contains much alcohol, heat until most of it has been removed; in other cases take out the wool after 5–15 min. and rinse with  $H_2O$ . Then treat the liquid with 3 or 4 drops of  $HCl$  for each 100 cc of soln and warm again for 10–20 min. with a clean piece of wool. If the wool takes up much coloring matter in either case, the presence of coal tar dyes is indicated.

The basic colors dye the fiber best from neutral or faintly ammoniacal solns and, if present, they will appear on the first piece of wool. Acid colors dye from neutral solns, but more readily from those containing free acid. The lichen colors<sup>2</sup> (archil, cudbear, litmus) go readily on wool, however, and many other natural colors, such as turmeric, will dye the fiber if present in large amount. On the other hand, a few

coal tar dyes, especially auramine O and naphthol green B, are quite unstable, and if present in small quantities may give no distinct dyeing. Acid dyes are much more frequently used than basic dyes, and in most cases they may be removed from wool without much decomposition by "stripping" the latter with  $\text{NH}_4\text{OH}$  (1+9).<sup>4</sup> Many natural colors are destroyed by the action of the alkali, while others remain for the most part on the fiber.

If the behavior with wool in neutral and acid solns indicates the presence of acid dyes, rinse the colored cloth thoroly with  $\text{H}_2\text{O}$ , cover with  $\text{NH}_4\text{OH}$  (1+9) in a casserole, and boil for a few min. Remove the cloth and squeeze out the adhering liquid. Boil the ammoniacal soln to remove the excess of  $\text{NH}_3$ , drop in a piece of clean wet wool, make distinctly but not strongly acid with  $\text{HCl}$  (1+9), and boil again. If acid coal tar dyes are present, they will usually give a fairly clean, bright dyeing on the second piece of wool. A further purification may be carried out by repeating the stripping and redyeing, tho this procedure is generally accompanied by a corresponding loss of dye.

(b) *Candies and similar colored sugar products*.—Dissolve about 20 g of the sample in 100 cc of  $\text{H}_2\text{O}$  and treat the soln as directed under (a). When the coloring matter is on the surface of the candy, pour off the soln before the colorless inner portion has dissolved.

(c) *Jams and jellies*.—Boil a mixture of 10–20 g of the sample and 100 cc of  $\text{H}_2\text{O}$  with wool in neutral and also in acid soln as directed under (a). For thick jams it is usually better, tho less easy, first to extract the coloring substances by treating the product as directed under (d).

(d) *Canned and preserved fruits and vegetables, sausage casings, smoked fish, coffee, spices, etc.*—Macerate 20–200 g of the sample with 4–5 times its weight of alcohol, 80% by volume. Allow to stand a few hours, pour off the solvent as completely as possible, and repeat the extraction, using alcohol 70% by volume and containing approximately 1% of  $\text{NH}_4\text{OH}$ . (1) Examine separately the filtered alcoholic extracts as directed under (a); or, (2) boil the ammoniacal soln until practically neutral, complete the neutralization with acetic acid, add the neutral 80% alcohol extract, continue the evaporation until most of the alcohol is removed, and boil a small portion with wool as directed under (a).

(e) *Cocoa and chocolate products*.—Treat cocoa as directed under (d). The alcoholic extract will contain large quantities of natural coloring matters, and several dyeings and strippings may be necessary to remove these in order to show the presence of coal tar dyes.

Chocolate may be treated similarly, but the following procedure is preferable: Wash 20–200 g of the well-divided sample with gasoline on a filter until most of the fat has been removed; if the gasoline is colored, reserve for the examination of oil-soluble dyes as directed under 3. Remove most of the adherent solvent from the residue by evaporation or pressure between layers of absorbent paper and digest with alcohol as directed under (d).

Coal tar dyes may also be detected in chocolate and cocoa products by mixing the samples directly with 3–4 times their weight of hot  $\text{H}_2\text{O}$  and immediately boiling the magma with wool, as directed under (a).

(f) *Cereal products (macaroni or other alimentary products)*.—Use 500 g of coarsely ground sample and proceed as directed under XX, 79.

### 3

#### *Oil-Soluble Coal Tar Dyes<sup>4</sup>*

Prepare an alcoholic soln of the dye by applying one of the following methods to

the oil or fat, obtained by extraction with ether or gasoline if the nature of the substance requires it:

(a) Shake the oil or melted fat with an equal volume of alcohol, 90% by volume, and wash the alcoholic extract with several portions of gasoline to free the coloring matter from foreign fats. The alcohol, after separation, will contain aniline yellow, butter yellow, aminoazotoluene, auramine, sudans, yellow OB, yellow AB, etc., if present.

(b) Saponify 20-200 g of the oil or fat with 0.5 *N* alcoholic KOH, remove most of the alcohol on the steam bath, and extract the soap with ether or gasoline. Remove the dyes from the solvent with 10 cc portions of a mixture containing 1 part of HCl and 5 parts of glacial acetic acid. Most of the common dyes are removed by this treatment, tho the digestion with strong alkali may cause some decomposition and make the extraction rather troublesome.

(c) Dilute 20-200 g of the oil or melted fat with 1-2 volumes of gasoline and shake out successively with 2-4% KOH or NaOH soln, HCl (1+3), and  $H_2PO_4$ - $H_2SO_4$  mixture, prepared by mixing 85%  $H_2PO_4$  with about 10-20% by volume of  $H_2SO_4$ . The dilute alkali extracts sudan G (10) [23] and annatto (709) [1241]. The dilute HCl extracts aniline yellow (7) [15], aminoazotoluene (-) [17], and butter yellow (16) [19], the first two forming orange-red, the latter cherry-red solns in this solvent. The  $H_2PO_4$  mixture is necessary for the extraction of sudan I (11) [24], sudan II (49) [73], sudan III (143) [248], and the homologue of the last, sudan IV (-) [258]. Benzeneazo-beta-naphthylamine (-) [22] and homologues also come in this group, tho they readily undergo chemical changes in the strongly acid mixtures. The procedure is not very suitable in the presence of auramine, but this dye is seldom found in oils. Neutralize the alkaline and the dilute HCl solns; dilute the  $H_2PO_4$  mixture and partially neutralize, cooling the liquid during this operation; and extract the dyes by shaking with ether or gasoline.

For the direct dyeing test use the alcoholic soln obtained as directed under (a). Evaporate to dryness the ether or gasoline solns obtained as directed under (b) and (c) and dissolve the residue in 10-20 cc of 95% alcohol. To the alcoholic soln add some strands of white silk and a little  $H_2O$  and evaporate on a steam bath until the alcohol has been removed or the dye is taken up by the silk. The dyeing test is sometimes unsatisfactory, and in all cases a small portion of the alcoholic soln should be tested by treating with an equal volume of HCl and  $SnCl_2$  soln. The common oil-soluble coal tar dyes are rendered more red or blue by the acid and are decolorized by the reducing agent. Most of the natural coloring matters become slightly paler with the acid and are little changed by the  $SnCl_2$  soln.

#### SEPARATION BY IMMISCIBLE SOLVENTS PROCEDURE<sup>6</sup>

##### 4 *Coal Tar Dyes in General*

The use of immiscible solvents for the separation of mixtures of coloring matters generally requires a systematic fractionation since many dyes do not differ very greatly in their solubilities in the various solvents.

##### 5 PREPARATION OF SOLUTION

(a) *Water-soluble colors*.—Proceed as directed under 2, omitting the fixation of the color on wool, and obtain an aqueous soln as free as practicable from suspended matter, alcohol, acids, alkalis, and salts. Liquids require no preparation except the removal of any alcohol that may be present.



(b) *Water-insoluble lakes*.—If the sample is in solid form, treat the well-divided material with sufficient  $H_2O$  to form a paste.

(c) *Oil-soluble dyes*.—Proceed as directed under 3, preferably 3(a) or 3(c).

## 6

*Basic Dyes*

Most basic dyes may be separated from mixtures by making alkaline with 10% NaOH soln and shaking with ether.<sup>7</sup> Use the sample prepared as directed under 5 for this purpose. Separate the ether layer, which may or may not be colored; wash it twice with a few cc of  $H_2O$  to remove excess of alkali; and shake with acetic acid (1+18), which will take up any dye present and form a colored soln. Altho this treatment may, to some extent, alter the common basic colors, it can be used for the detection of methyl violet B (451) [680], magenta (448) [677], bismarck brown (197) [331], malachite green (427) [657], and rhodamine B (504) [749]. With care auramine (425) [655] also may be separated in this way, tho it is quickly decomposed on standing in alkaline soln.

## 7

*Acid Dyes*

The following short procedure is often convenient for the examination of mixtures of acid dyes: Make the prepared sample, 5, strongly acid by adding  $\frac{1}{2}$  its volume of HCl and shake with amyl alcohol. Separate the amyl alcohol soln and wash by shaking with successive portions of  $\frac{1}{2}$  its volume of  $H_2O$ , reserving the portions in separate test tubes or beakers. Because of the varying acid content of the amyl alcohol these washings will show a regular decrease in acidity, and the coloring matters will appear in maximum quantity in the different fractions according to their respective solubilities. Ponceau 6R (108) [186] is washed out chiefly while the acidity is still high, approximately normal. Amaranth (107) [184], brilliant scarlet (106) [185], tartrazine (94) [640], sunset yellow FCF, orange G (14) [27], and soluble blue (480) [707] appear when the washings have an acidity of about 0.25 *N*, and palatine scarlet (53) [77], ponceau 2R (55) [79] and 3R (56) [80], ponceau SX, naphthol yellow S (4) [10], cochineal (706) [1239], crystal ponceau (64) [89], and azorubine A (103) [179] between  $\frac{1}{16}$  *N* and  $1/256$  *N*. When practically all the acid is removed, orange I (85) [150], orange II (86) [151], and croceine orange (13) [26], begin to wash out, and less readily, orange IV (88) [143] and metanil yellow (95) [138]. Finally the unsulfonated coloring matters, such as erythrosine G (516) [772], erythrosine B (517) [773], and the rose bengals (520) [777] and (523) [779] are removed very slowly by  $H_2O$  or not at all unless the solvent is diluted with gasoline and the dyes are removed with  $H_2O$  containing a few drops of  $NH_4OH$ . Acid yellow (8) [16] and brilliant yellow S (89) [144] are not very uniform in composition. They are partially taken up by amyl alcohol from acid soln and appear chiefly in the first washings. Indigotine (692) [1180] behaves somewhat similarly.

When it appears probable that only the coal tar dyes listed in the regulations for the enforcement of the Federal Food and Drugs Act<sup>8</sup> for use in food products are present, the following abridged procedure may be conveniently used for their separation:

**PERMITTED COAL TAR FOOD COLORS<sup>9</sup>**

(Amaranth, ponceau 3R, ponceau SX, erythrosine, orange I, light green SF yellowish, fast green FCF, guinea green B, brilliant blue FCF, indigotine, naphthol yellow S, sunset yellow FCF, tartrazine, yellow AB, and yellow OB.)

## 8

**PREPARATION OF SOLUTION**

(a) *For foodstuffs containing oil-soluble dyes*.—Proceed as directed under 3(a),

evaporate the 90% alcoholic extract to dryness in a casserole, treat the residue with 40 cc of low-boiling gasoline, and shake the gasoline soln with 2 or 3 portions of 5 cc each of 2-4% NaOH soln (to remove annatto, turmeric, etc., if present). The gasoline soln will contain the yellow OB and yellow AB.

(b) *For foodstuffs which contain no oil-soluble dyes or from which these dyes have been removed.*—Proceed as directed under 2, omitting the fixation of the color on wool, and obtain an aqueous soln as free as possible from suspended matter, alcohol, acids, alkalies, and salts. The dye soln should be preferably between 0.01 and 0.05%. The soln obtained in the examination of colored food products rarely requires further dilution, but with commercial food colors care must be taken that the concentration is not too great.

## 9

## SEPARATION

(a) *Yellow AB and yellow OB.*—Extract the gasoline soln of these dyes, 8(a), 3 times with  $\frac{1}{2}$  its volume of 13 N  $\text{H}_2\text{SO}_4$ . Shake each acid extract successively with 2 portions (equal volumes) of low-boiling gasoline, using the same 2 portions of gasoline for each acid portion. Extract each of the 2 latter gasoline portions with 20 cc of 13 N  $\text{H}_2\text{SO}_4$ , using the same acid portion successively for both gasoline portions. Finally extract the second of these gasoline portions with another 20 cc portion of 13 N  $\text{H}_2\text{SO}_4$ . (The original gasoline soln has now been shaken with acid 3 times, the next gasoline portion 4 times, and the third 5 times.) Combine the acid extracts, dilute with  $\text{H}_2\text{O}$ , re-extract with low-boiling gasoline, and evaporate the solvent. Yellow AB will be found in a practically pure state. Combine the gasoline solns (original and subsequent solns left after the acid washings), wash with small portions of  $\text{H}_2\text{O}$  to remove excess of acid, and evaporate the solvent. The yellow OB will remain as a residue. (This method is not absolutely quantitative, but it is sufficiently accurate to make a separation of either of the dyes with comparatively little contamination from the other.) The following color test may be applied to the separated dyes to confirm their identity: Shake 5 cc of a neutral gasoline soln of the dye in a test tube with 5 cc of a mixture of 1 part of 40%  $\text{HCHO}$  soln and 4 parts of acetic anhydride. Both coloring matters are extracted by the acetic anhydride, yellow AB giving in a few seconds a red colored soln, and yellow OB, under the same conditions, giving an orange colored soln.

(b) *Amaranth, ponceau 3R, ponceau SX, erythrosine, orange I, light green SF yellowish, fast green FCF, guinea green B, brilliant blue FCF, indigotine, naphthol yellow S, sunset yellow FCF, and tartrazine.*—To the soln obtained under 8(b), add sufficient 25% salt soln to make the concentration about 10% and 1 part acetic acid to every 7 parts of soln. Extract with 3-50 cc portions of amyl alcohol. Draw off the lower layer and reserve for further treatment. Wash the amyl alcohol extract in rotation with 25 cc portions of 5% salt soln until the washings are colorless or nearly so. Add the washings to the original aqueous soln. Dilute the amyl alcohol extract with an equal volume of gasoline and wash with 25 cc portions of  $\text{H}_2\text{O}$  until all color is extracted. The coloring matters obtained are orange I and guinea green B. For their separation see (1) below. Treat the amyl alcohol gasoline soln with 10 cc portions 0.1 N NaOH or with 10 cc portions of  $\text{NH}_4\text{OH}$  (1+9), which will remove erythrosine. The original soln and washings (from which the 3 named dyes were removed) are acidified with HCl (1 volume acid to 40 volumes of soln) and extracted in 50 cc volumes with three 50 cc portions of amyl alcohol. Reserve the lower aqueous layer for further treatment. Wash the amyl alcohol extract with 25 cc portions of 0.25 N HCl until the washings are colorless or nearly so. Combine washings with the aqueous soln above. The amyl alcohol is extracted with several 25 cc

portions of  $H_2O$  until all color is extracted. The coloring matters obtained are ponceau 3R, ponceau SX, and naphthol yellow S. For their separation see (2). The original soln and washings (from which the 6 named dyes were removed) are treated in 50 cc volume with 3–50 cc portions of  $\alpha$  dichlorhydrin. Reserve the upper aqueous layer for further treatment. Wash the dichlorhydrin extract in rotation with several 20 cc portions of 25% salt soln. Combine washings with the aqueous soln above. Dilute the dichlorhydrin extract with 2 volumes of  $CCl_4$  and extract with several 25 cc portions of  $H_2O$  until all color is extracted. The coloring matters obtained are light green SF yellowish, fast green FCF and brilliant blue FCF. For their separation see (3). Further acidify the original soln and washings (from which the 9 named dyes were removed) with HCl (1 vol. acid to 40 vol. soln) and extract in 50 cc volumes with three 50 cc portions of amyl alcohol. (If the color intensity of the soln was not too strong, all coloring matter should have been extracted by the solvent.) Discard the lower colorless or nearly colorless layer and wash out the dyes from the amyl alcohol extract in rotation with several 25 cc portions of  $H_2O$ , until all color is extracted. The coloring matters obtained are indigotine, amaranth, tartrazine, and sunset yellow FCF. For their separation see (4).

(1) *Orange I and guinea green B.*—Extract the combined colors with two 20 cc portions of  $\alpha$  dichlorhydrin. Discard the colorless upper aqueous layer, dilute the solvent with 2 volumes of  $CCl_4$ , and extract out orange I in rotation with several 10 cc portions of  $H_2O$ , and guinea green B with several 10 cc portions of 25% alcohol.

(2) *Ponceau 3R, ponceau SX, and naphthol yellow S.*—Acidify the combined colors with HCl (1 part acid to 10 parts of soln) and extract the naphthol yellow S with two 20 cc portions of washed ethyl acetate or amyl acetate. Ponceau 3R and ponceau SX are not extracted appreciably and remain in the aqueous layer. Wash the solvent with 5 cc portions of  $N$  HCl to remove traces of the ponceaus. Naphthol yellow S is removed from the combined ethyl acetate or amyl acetate with 5 cc portions of  $NH_4OH$  (1+9). Extract the remaining ponceau soln with 20 cc portions of amyl alcohol and wash out excess of acid twice with a few cc portions of  $H_2O$ . Dilute amyl alcohol with an equal volume of gasoline, and remove the color with small volumes of  $H_2O$ . Treat 10 cc of this soln with 1 cc of HCl, 2 cc of strong bromine water, and lastly 3 cc of saturated hydrazine sulfate soln and immediately pour into a test tube containing 10 cc of 2  $N$  sodium carbonate and 2 drops of 1% alcoholic  $\alpha$  naphthol. (A light orange soln indicates ponceau 3R. A deep brownish red soln indicates ponceau SX.) Add to the soln 5 cc of ether, mix well and draw off the lower aqueous layer which, if colored, contains ponceau SX. To the ethereal extract add an equal volume of HCl when the formation of a purplish solution confirms the presence of ponceau 3R.

(3) *Light green SF yellowish, fast green FCF, and brilliant blue FCF.*—Treat the combined colors with an equal volume of 2  $N$   $Na_2CO_3$  soln and extract in 25 cc volumes with two 50 cc portions of  $N$  butyl alcohol. Draw off the lower aqueous layer containing the fast green FCF and wash out the last traces from the solvent with 25 cc portions of 2  $N$   $Na_2CO_3$ . Reserve washings and add to the aqueous soln for confirmatory tests. Light green SF yellowish is colorless in the solvent while brilliant blue FCF imparts a bluish green to it. To prove the presence of light green SF yellowish in presence of brilliant blue FCF proceed as follows: Dilute the solvent with an equal volume of gasoline and remove color with small portions of  $H_2O$ . Treat 20 cc of soln with 4 cc of 10% NaOH and boil for 5 min. Brilliant blue FCF is changed to a red phase, while light green SF yellowish is changed to a yellow.

Acidify with 10 cc of glacial acetic acid, which changes brilliant blue FCF to a violet and light green SF yellowish to a green. Treat with about 3 g of zinc dust and heat until soln is decolorized. Filter, make slightly alkaline with  $\text{NH}_4\text{OH}$  and later make acid with acetic acid and bring to a boil. In the presence of light green SF yellowish a deep green soln is formed while brilliant blue FCF remains colorless.

(4) *Indigotine, amaranth, tartrazine, and sunset yellow FCF*.—To separate the indigotine heat a small portion of the soln, which should be neutral or faintly acid, to boiling, and add a few crystals of  $\text{Na}_2\text{S}_2\text{O}_4$  until all the dyes are reduced. On adding a few drops of glacial acetic acid and shaking with air the indigotine is quickly restored, while amaranth, tartrazine, and sunset yellow FCF are destroyed. If a positive test for indigotine is obtained, add to the remainder of the mixed dye soln several decigrams of urea, heat, and while the mixture is boiling add 1 or 2 drops of 10%  $\text{NaNO}_2$ . Indigotine is converted to the pale yellow isatine sulfonate, while amaranth, tartrazine, and sunset yellow FCF are but little affected. Acidify the resultant mixture with  $\text{H}_2\text{SO}_4$  (1+4), using 1 part of dilute acid to 10 parts of soln. Extract in 25 cc portions with three 50 cc portions of *N* butyl alcohol. Draw off lower layer and pass successively thru all the funnels. Reserve the aqueous layer if colored; if not colored, discard. Prepare the following soln: 13.5 cc of  $\text{H}_2\text{SO}_4$ , 100 g of anhydrous  $\text{Na}_2\text{SO}_4$ , and sufficient  $\text{H}_2\text{O}$  to make 1 liter. Extract the *N* butyl alcohol successively with 25 cc portions of the soln until washings are colorless. Reserve them for amaranth and tartrazine. Dilute the *N* butyl alcohol with an equal volume of gasoline and remove sunset yellow FCF with  $\text{H}_2\text{O}$ . Confirm with dyeing tests and wet reactions.

Acidify the reserved soln with  $\text{HCl}$  (1 vol. acid to 20 of soln) and extract with two 30 cc portions of amyl alcohol. This will extract both amaranth and tartrazine while the isatine compound, being less readily extracted, remains in the lower layer and is discarded. Remove the coloring matter with several 10 cc portions of  $\text{H}_2\text{O}$ . To a portion of the soln add 5 drops of  $\text{NH}_4\text{OH}$  and a few crystals of  $\text{Na}_2\text{S}_2\text{O}_4$ . (This treatment will destroy amaranth completely, leaving tartrazine practically unaltered.) Add an excess of  $\text{HCl}$  and speedily extract the dye with a small volume of amyl alcohol, from which soln tartrazine can be removed with 0.25 *N*  $\text{HCl}$ . Treat another 10 cc portion of the neutral dye soln in a test tube with 2 cc of 20%  $\text{NH}_4\text{Cl}$  and 1 cc of 25%  $\text{KCN}$  soln and heat in a boiling water bath for 5 min. Cool rapidly and acidify with 2 cc of  $\text{HCl}$  and extract with 10 cc of amyl alcohol (caution). Draw off the lower layer and discard. Remove tartrazine with 5 cc portions of 0.25 *N*  $\text{HCl}$ ; amaranth is converted to a lower sulfonated dye, and is not removed at that acid concentration. Dilute the solvent with an equal volume of gasoline and extract the dye with small volumes of  $\text{H}_2\text{O}$  (amaranth is modified to a brownish red dye).

## 10

IDENTIFICATION<sup>10</sup>

The most widely used tests for the identification of coal tar dyes refer to the changes produced with acids and alkalis. Other tests, based upon the behavior with reducing agents, followed perhaps by treatment with oxidants or by separation and identification of the reduction products,<sup>11</sup> and tests based upon oxidation of the dye and treatment of the oxidation products,<sup>12</sup> are generally applicable. Spectroscopic methods are also used.<sup>13</sup>

## 11

*I. By Color Changes Produced with Acids and Alkalies*

Transfer the separated coloring matter to wool (or to silk in the case of oil-soluble dyes) by boiling as directed under 2(a) or 3. (Care should be taken that the

TABLE 1.—Color reactions produced on dyed fibers by various reagents

COLORING MATTER	C. I. NO.	S. & J. NO.	STRONG HYDROCHLORIC ACID	CONCENTRATED SULFURIC ACID	10% SODIUM HYDROXIDE SOLUTION	SOLUBLE AMMONIUM HYDROXIDE
Rhodamine B	749	504	Orange	Yellow	Blue	Blue
Rose Bengal	779	523	Almost decolorized	Orange	No change	No change
Achil	1242	710	Red	Reddish brown	Violet	Violet
Magenta	677	448	Yellowish brown	Yellowish brown	Decolorized	Paler
Acid Magenta	692	462	Almost decolorized	Yellow	Dull brown	Decolorized
Palatine Red	85	62	Darker	Blue	Brick red	Little change
Bordeaux B	88	65	Violet	Blue	Dull brownish to orange red	Little change
Amaranth	184	107	Slightly darker	Violet to brownish	Red	Little change
Azorubine A	179	103	Little change	Violet	No change	Red
Erythrosine	778	517	Orange-yellow	Orange-yellow	Dull violet-red	Little change
Ponceau 6RB	280	169	Blue	Blue	Brown	Orange-red
Ponceau 6R	186	108	Violet-red	Violet	Dull brown	Little change
Crystal Ponceau	89	64	Red	Little change	Dull orange	Orange yellow
Ponceau 3R	80	56	Deeper red	Deeper red	Orange yellow	Orange yellow
Ponceau SX	...	...	Violet, then brown	Violet-red	Violet-red	Little change
Sudan III*	...	143	Greenish blue	Green	Red	Red
Safranine	248	584	Red	Violet-red	Yellowish brown	Orange-red
Brilliant Scarlet	841	106	Little change	Little change	Brownish yellow	No change
Ponceau 2R	79	55	Darker	Violet-red	Brownish yellow	No change
Palatine Scarlet	77	53	Yellow-orange	Yellow-orange	No change	No change
Erythrosine G	772	516	Red	Violet-red	Little change	No change
Sudan II*	73	49	Orange-red	Red	Redder	No change
Sudan I*	24	11	Little change	Little change	Violet-red	Violet-red
Cochineal	1239	706	Redder, darker	Browner	Yellow	Yellow
Bismarck Brown	331	197	Redder, darker	Browner	Yellow	Yellow
Bismarck Brown R	332	201	Redder, darker	Violet	Red, dark	Red, dark
Orange I	150	85	Violet	Red	Dull red	No change
Orange II	151	86	Red	Orange	Slightly darker	No change
Croceine Orange	26	13	Little change	Orange	Dull, brownish red	No change
Orange G	27	14	Red	Violet	Little change	No change
Ordnanceazobeta-naphthylamine* (Yellow OB)	61	...	...	...	...	...

\* Oil-soluble.

	22	...	Red	Violet	Little change	No change
Benzenesazobeta-naphthylamine* (Yellow AB)	22	...	Orange-yellow	Brownish yellow	Orange-yellow	No change
Sudan G*	23	10	Violet-red	Orange-yellow	No change	No change
Butter Yellow*	19	16	Violet-red	Orange-yellow	Little change	No change
Aniline Yellow*	15	7	Dull orange	Orange-yellow	Little change	No change
Aminoazoorthotoluene*	17	...				
Fluoresceine	766	510	Little change	Little change	Green fluorescent	Green fluorescent
Metanil Yellow	138	95	Violet-red	Violet	No change	No change
Azoflavine	145	92	Violet-red	Violet-red	Dull brown	Little change
Acid Yellow	18	8	Red	Orange	Little change	No change
Brilliant Yellow S	144	89	Violet-red	Violet-red	Little change	Little change
Tartrazine	640	94	Slightly darker	Slightly darker	Browner	Little change
Sunset yellow FCF	...	...	Slightly redder	Slightly redder	No change	No change
Naphthol Yellow S	10	4	Almost decolorized	Very pale, dull brown	No change	No change
Auramine	655	425	Decolorized	Almost decolorized	Decolorized	Paler
Turmeric	1238	707	Red	Reddish brown	Orange	Orange
Quinoline Yellow	801	667	Slightly darker	Brownish yellow	Slightly paler	Little change
Naphthol Green B	5	398	Yellowish	Brownish yellow	No change	No change
Guinea Green B	666	433	Pale orange-yellow	Yellowish brown	Decolorized	Decolorized
Light Green SF Yellowish	670	435	Pale orange-yellow	Yellowish brown	Decolorized	Decolorized
Fast Green FCF	...	...	Orange	Green to brown	Blue	Blue
Brilliant Blue FCF	...	...	Yellow	Yellow	No change	No change
Night Green 2B	667	438	Pale orange-yellow	Yellowish brown	Decolorized	Paler
Malachite Green	657	427	Almost decolorized	Almost decolorized	Decolorized	Decolorized
Erioglaucine A	671	436	Yellow	Pale, dull yellow or brown	Slightly darker	Little change
Patent Blue A	712	442	Pale orange-yellow	Green to brown	Little change	Little change
Soluble Blue	707	480	Paler	Brown	Pale reddish	Almost decolorized
Indigotine	1180	692	Slightly darker	Darker	Greenish yellow	Greenish blue
Fornyl Violet	698	468	Pale orange-yellow	Pale, dull orange	Decolorized	Decolorized
Methyl Violet	680	451	Yellowish	Yellowish	Decolorized	Almost decolorized
Nigrosine, soluble	865	602	Dull bluish	Dull greenish	Brownish red, paler	Pale reddish

final dyeing is made in a soln fairly free from foreign matter such as sugar or aromatic substances, which, adhering to the fiber, may modify the reaction. In most cases the quantity of color available is small and should not be used to dye too large a piece of wool, or silk.) Rinse the dyed fiber thoroly in running  $H_2O$ , dry, cut into small pieces, and place separately in the depressions of a white porcelain spot plate. Moisten the pieces with  $HCl$ ,  $H_2SO_4$ , 10% soln of  $NaOH$ , and  $NH_4OH$  containing 12% by weight of  $NH_4$ . (For many coloring matters the hue upon treatment with acids or alkalies varies markedly with the concentration of the reagents and quantity of dye present; therefore the unknown dye should be compared with dyeings of known colors of approximately the same dye concentration as shown by their appearance.)

Table 1 shows the color changes produced on wool dyed with 0.1–0.5% solns of the respective coloring matters. Included also are the reactions of the oil-soluble colors, but these refer to dyeing on silk. The dyes are arranged approximately according to hue. Brown is classed with orange; black (gray), with violet.

## 12

## II. By Special Tests

(a) *Oil-soluble dyes (yellow AB and yellow OB).*—A method of separating and identifying the 2 permitted oil-soluble dyes is given under 9(a). The alcoholic solns of these dyes become red on treatment with  $HCl$ ; are unaffected by alkalies; are reduced by  $SnCl_2$ ,  $TiCl_3$ , and  $Na_2S_2O_4$ ; and the color is not restored to the reduced solns on the addition of  $FeCl_3$  or  $K$  persulfate.

(b) *Water-soluble dyes (amaranth, ponceau 3R, ponceau SX, erythrosine, orange I, light green SF yellowish, fast green FCF, guinea green B, brilliant blue FCF, indigotine, naphthol yellow S, sunset yellow FCF, and tartrazine).*—Treatment of these dyes in acid soln with  $SnCl_2$ ,  $TiCl_3$ ,  $Zn$  dust, or  $Na_2S_2O_4$  decolorizes indigotine, amaranth, ponceau SR, ponceau SX, orange I, sunset yellow FCF, and tartrazine. With indigotine the color returns on shaking with air, but more readily on warming or on the addition of  $FeCl_3$  or  $K$  persulfate. Excess of the reducing agents must be avoided. With the last 6 named dyes the color is not restored. Dilute solns of light green SF yellowish, guinea green B, fast green FCF, brilliant blue FCF, naphthol yellow S, and erythrosine become yellow or colorless with acid so that the effects of acid-reducing agents are not so readily apparent. Neutral solns of naphthol yellow S are first changed to pink and later decolorized by  $Na_2S_2O_4$  and other reducing agents, the color not returning with air or oxidants. Erythrosine, light green SF yellowish, fast green FCF, brilliant blue FCF, and guinea green B become paler with  $Na_2S_2O_4$ , the color being partially restored upon the addition of  $K$  persulfate.

In hot solns containing an excess of  $Na$  tartrate, the water-soluble dyes are readily decolorized by  $TiCl_3$ .<sup>14</sup> In the case of indigotine, if the reducing agent has been added carefully and an excess avoided, the blue color readily returns on shaking with air. With erythrosine, light green SF yellowish, fast green FCF, brilliant blue FCF, and guinea green B the color is scarcely restored by air, but on cooling and adding  $K$  persulfate it returns imperfectly. The reduction products of the other dyes do not give colored solns again on oxidation, if a slight yellowish or brownish tint that may sometimes appear is disregarded.

(1) *Light green SF yellowish, fast green FCF, brilliant blue FCF, and guinea green B* belong to the triphenyl-methane type of dyes. Solns of light green SF yellowish and guinea green B behave similarly with acids, alkalies, and reducing agents, producing a yellow to a greenish yellow with mineral acids, and an almost colorless soln with alkalies as well as with reducing agents. On the other hand, while the

reactions of fast green FCF and brilliant blue FCF are similar with acids and reducing agents, they differ in respect to their behavior to alkalis. While light green SF yellowish is decolorized by the addition of  $\text{NH}_4\text{OH}$  or 10%  $\text{NaOH}$ , fast green FCF produces a deep blue soln by similar treatment, which is not altered even on boiling; brilliant blue, on the other hand, is not affected by  $\text{NH}_4\text{OH}$  or fixed alkalis in the cold, but is changed to a reddish purple soln upon boiling with 10%  $\text{NaOH}$ . The easy solubility of these 4 colors in  $\alpha$  dichlorhydrin differentiates them from all other permitted dyes. To separate guinea green B from light green SF yellowish, fast green FCF and brilliant blue FCF, proceed as follows:

*Light green SF yellowish and guinea green B.*—Prepare a soln of 250 g of  $\text{NaCl}$ , 27 g of crystallized Na acetate, and 24 cc of acetic acid in  $\text{H}_2\text{O}$ , and dilute to 1 liter.

To separate and differentiate the 2 green coloring matters add to every 20 cc of dye soln 1 cc of  $\text{HCl}$  and extract with an equal volume of amyl alcohol. Draw off the lower layer and remove the light green SF yellowish by washing the remaining amyl alcohol portion with equal volumes of the  $\text{NaCl}$ -sodium acetate soln until no more color is extracted. Dilute the amyl alcohol with an equal volume of gasoline and remove the guinea green B with  $\text{H}_2\text{O}$ .

*Light green SF yellowish, fast green FCF, and brilliant blue FCF.*—To separate and differentiate proceed as directed under 9(3).

(2) *Indigotine* is extracted in small proportions from slightly acid solns by shaking with  $\alpha$  dichlorhydrin, from which it may be removed with small portions of 25% salt soln. Most of the other common bluish dyes are triphenyl-methane derivatives and are relatively more soluble in the solvent than in the aqueous layer. Indigo is readily destroyed by boiling with a very small amount of a fixed alkali soln, by which treatment it may be readily eliminated from other coloring matters.

(3) *Ponceau 3R* gives in neutral or faintly acid solns a bluish red, flocculent precipitate with  $\text{BaCl}_2$  or Ba acetate, practically all the dye being removed from soln. Some of the soln obtained in the separation, 9, may be used in this test, the free  $\text{HCl}$  first being neutralized with Na acetate; or better, it may be evaporated to dryness on a steam bath to remove the acid and the residue taken up with a little  $\text{H}_2\text{O}$ . A brick red precipitate will be formed on standing, when a neutral soln of the dye is treated with a 20% soln of neutral Pb acetate. The soln should contain 0.005% or more of the dye.

(4) *Naphthol yellow S*, in solns containing an excess of  $\text{NH}_4\text{OH}$  or  $\text{Na}_2\text{CO}_3$ , becomes intensely rose-red on the addition of  $\text{Na}_2\text{S}_2\text{O}_4$ , the color gradually fading again as complete reduction takes place. A red coloration is also produced if an aqueous soln of the dye is treated with a few drops of 40%  $\text{SnCl}_2$  and an excess of 20%  $\text{KOH}$  is added.

(5) *Tartrazine* is characterized by its comparative inactivity towards acids and alkalis, the soln of the dye being hardly altered by the reagents. An alkaline soln of the dye is reduced with  $\text{Na}_2\text{S}_2\text{O}_4$  only with difficulty. A concentrated neutral or slightly acid soln of the dye, when reduced with  $\text{SnCl}_2$  soln or Zn dust and made slightly alkaline and filtered, will develop a purple coloration on standing.

(6) *Orange I* can readily be recognized by its behavior toward reagents. With a large excess of  $\text{HCl}$  it produces a purplish-red; with alkali in large excess it produces a bright red soln.

(7) *Erythrosine* differs from most of the common dyes in that it contains 1. To test for 1, acidify the soln with  $\text{H}_2\text{SO}_4$ , shake with ether, separate the ether soln of the color, and evaporate to dryness in a Pt dish after the addition of a few drops of  $\text{Na}_2\text{CO}_3$  soln or sufficient to form the deep red Na salt. Hold the dish containing the



residue in the Bunsen flame until organic matter is destroyed, take up the residue with  $H_2O$ , acidify with  $H_2SO_4$ , and test for I in one of the usual ways, such as with Cl water and  $CS_2$  or  $CCl_4$ , or with starch paste and an oxidizing agent. It is useless to test for I with very small quantities of dye, but in most cases sufficient coloring matter can be separated from the food product to give satisfactory results.

## 13

## NATURAL COLORING MATTERS

As a class the natural coloring matters show much less tendency to dye animal fiber than do the common synthetic colors. In many cases the crude products used contain a number of colored substances, and a complete separation is not practicable. As dilute solns of most of the natural coloring matters are sensitive to alkalis, and some are sensitive to acids, such reagents must be used with care. Relatively few good tests are known for the common natural colors. Some of their most useful analytical properties<sup>15</sup> are given in Table 2.

The properties of pure preparations of the various natural coloring matters are described, for the most part, by Rupe,<sup>16</sup> and by Perkin and Everest,<sup>17</sup> reference being made in these works to the original literature. Properties of the chlorophylls and carotinoids are given by Willstätter and Stoll,<sup>18</sup> those of the coloring matters of the cornflower, rose, pelargonia flower, larkspur, cranberry, whortleberry, purple grape, sloe, cherry, plum, radish, and red beet by Willstätter and coworkers.<sup>19</sup>

## 14

## SEPARATION

(a) *By extraction with ether from neutral solns.*—From neutral solns ether extracts carotin, xanthophyll (the pigments found in leaves, fats and oils, egg yolk, carrots, etc.), the coloring matter of tomatoes and paprika, and green chlorophyll. The coloring matter remains in the ether soln on shaking with normal NaOH soln or normal HCl, no apparent change taking place, altho chemically the substances may be altered more or less by this treatment.

(b) *By extraction with ether from acid solns.*—From slightly acid solns ether extracts very readily and completely the coloring matter of alkanet, annatto, turmeric, and the red dyewoods, sandalwood, camwood, and barwood. It extracts in large proportions the flavone coloring matters of fustic, Persian berries and quercitron (after hydrolysis), as well as the coloring matter of Brazilwood and the green derivatives formed from chlorophyll by alkaline treatment. It extracts in relatively small quantity the coloring matters of logwood, archil, saffron, and cochineal. The coloring matters of this group are readily removed from ether by shaking with alkaline solns, but in most cases they rapidly undergo chemical change.

(c) *By extraction with amyl alcohol from acid solns.*—From slightly acid solns amyl alcohol extracts largely the coloring matters of logwood, archil, saffron, and cochineal. Amyl alcohol extracts in relatively small proportions caramel and the anthocyanins constituting the red coloring matter of the most common fruits.

## IDENTIFICATION

## 15

## I. By Color Changes Produced with Various Reagents

Evaporate to dryness the ether solns obtained under 14(a) and 14(b), warm the residue with a little alcohol, and dilute with  $H_2O$ . Dilute the amyl alcohol soln obtained under 14(c) with gasoline and extract with  $H_2O$ . To portions of these somewhat purified solns of the coloring matter apply the reagents in the following manner:

*Hydrochloric acid.*—Add to the soln, first 1 or 2 drops of strong HCl, then a large excess equal to 3-4 times the volume of the soln.

*Sodium or potassium hydroxide.*—Make the soln slightly alkaline by adding a drop of 10% NaOH or KOH soln.

*Sodium hyposulfite.*—Add a small crystal of  $\text{Na}_2\text{S}_2\text{O}_4$ .

*Ferric chloride.*—Add a small quantity of freshly prepared 0.5%  $\text{FeCl}_3$  soln very carefully, a small drop at a time, as the colorations are not obtained in some cases when an excess is used.

*Alum.*—Add to the test soln  $\frac{1}{2}$  its volume of 10% K- or  $\text{NH}_4$ -alum soln.

*Uranium acetate.*—Add 5% U acetate soln dropwise.

*Sulfuric acid on the dry color.*—Evaporate a small quantity of the soln or of the coloring matter in a porcelain dish. Cool thoroly and treat the dry residue with 1 or 2 drops of cold  $\text{H}_2\text{SO}_4$ . The colorations are in some cases extremely transitory, and they may be observed only the instant the acid wets the residue.

Table 2 shows the behavior of certain of the natural coloring matters when treated in the manner described above.

## 16

## II. By Special Tests

(a) *Chlorophyll.*—The "brown phase reaction"<sup>20</sup> may be useful for the characterization of chlorophyll, when this has not been previously treated with alkalis. Treat the green ether or petroleum ether soln of the coloring matter with a small quantity of 10% soln of KOH in methyl alcohol. The color becomes brown, quickly returning to green.

(b) *Annatto.*<sup>21</sup>—Pour on a moistened filter an alkaline soln of the color obtained by shaking out the oil or melted fat with warm 2% NaOH soln. If annatto is present, the filter paper will absorb the color, so that when washed with a gentle stream of  $\text{H}_2\text{O}$  it will remain dyed a straw color. Dry the filter and add a drop of  $\text{SnCl}_2$  soln. If the color turns pink, the presence of annatto is confirmed.

(c) *Turmeric.*—Treat an aqueous or dilute alcoholic soln of the color with HCl until the shade just begins to appear slightly orange. Divide the mixture into two parts and add some  $\text{H}_3\text{BO}_3$  powder or crystals to one portion. A marked reddening will be quickly apparent, best seen by comparison with the portion to which the  $\text{H}_3\text{BO}_3$  has not been added. The test may also be made by dipping a piece of filter paper in the alcoholic soln of the coloring matter, drying at  $100^\circ$ , then moistening with a weak soln of  $\text{H}_3\text{BO}_3$  to which a few drops of HCl have been added. On drying again a cherry-red color will be developed.

(d) *Cochineal.*—When the presence of cochineal is suspected, acidify the mixture with  $\frac{1}{2}$  its volume of HCl and shake with amyl alcohol. Wash the amyl alcohol soln of the coloring matter 2-4 times with equal volumes of  $\text{H}_2\text{O}$  to remove HCl, etc. Dilute the amyl alcohol with 1-2 volumes of gasoline and shake with a few small portions of  $\text{H}_2\text{O}$  to remove the color. Divide the combined aqueous extracts into 2 portions. To the first add, dropwise, 5% U acetate soln, shaking thoroly after each addition. In the presence of cochineal a characteristic emerald-green color is produced.<sup>22</sup> The green coloration with U salts is not developed in the presence of much free acid. Therefore, add a little Na acetate before making this test, or a correspondingly large quantity of U acetate must be added. To the second portion add 1 or 2 drops of  $\text{NH}_4\text{OH}$ ; in the presence of cochineal, a violet coloration results. This, however, is not so characteristic as the first test as many fruit colors give almost identical reactions. Cochineal is not decolorized by  $\text{Na}_2\text{S}_2\text{O}_4$  either in an acid, neutral or alkaline soln (differs from orchil).

TABLE 2.—*Reaction of certain natural coloring matters to common reagents*

COLORING MATTER	STRONG HYDROCHLORIC ACID	10 PER CENT SODIUM HYDROXIDE SOLUTION	SODIUM HYPOSULFITE	0.5 PER CENT FERRIC CHLORIDE SOLUTION	10 PER CENT ALUM SOLUTION	5 PER CENT URANIUM ACETATE SOLUTION	CONCENTRATED SULFURIC ACID ON DRY COLOR
Logwood	Deep red with excess of acid	Violet to violet-blue	Almost decolorized, color returning imperfectly by reoxidation	Dark shades of violet, brown or black (the first hue often evanescent)	Rose-red (change rather slow)	Violet, quickly fading	Red, changing to yellow
Red woods (Brazilwood, Sandalwood, Camwood and Barwood)	Deep red with excess of acid	Violet-red		Dark shades of violet, brown or black (the first hue often evanescent)	Rose-red (change rather slow)		
Anthocyanins of red fruit colors		Change to green, dull blue or slate color, usually very quickly becoming brown by oxidation	Anthocyanins derived by hydrolysis, almost completely decolorized				
Alkanet		Deep blue				Yellowish green	Violet-blue
Archil	Little or no change	Blue	Decolorized, color returning when shaken with air. Reaction more easily seen in alkaline solution				Violet-blue
Cochineal	Little or no change	Violet	No marked change	Slightly darker		Green	
Annatto	Remains orange. Little change		Little affected	No marked change. Perhaps somewhat browner			Blue
Turmeric (solution in ether or alcohol characterized by pure yellow color and light green fluorescence)	Orange-red or carmine-red on addition of several volumes of concentrated acid	Orange-brown	Little affected	No marked change. Perhaps somewhat browner	Little change	Somewhat browner	Red
Flavone colors of fustic, Persian berries, quercitron, etc.	Becomes intensely yellow with 2-4 volumes of concentrated acid	Bright yellow	Little affected	Olive-green or black colorations	More strongly yellow; fustic, developing a green fluorescence	Orange colorations	Yellow to orange
Saffron	Little or no change	Remains yellow	Little affected	No marked change. Perhaps somewhat browner	Little change	Not affected	Blue
Carotin and Xanthophyll	Little change. Perhaps slightly paler	Little or no change	Little affected				Blue, reaction obtained with difficulty
Green Chlorophyll	More brownish	"Brown phase reaction," 16(a)					
Caramel	Little or no change	Little change or slightly deeper brown	Slightly paler	No change			

As cochineal lakes often contain tin, further examination for this metal should always be made when water-insoluble cochineal compounds seem to be present.

(e) *Orchil*.—This coloring matter is either sulfonated or unsulfonated. Unsulfonated orchil is readily extracted by amyl alcohol from a weak acid soln, while the extraction of the sulfonated color is incomplete even from a strongly acidified soln. The behavior of the color towards acids and alkalies is similar to cochineal, e.g., HCl produces a yellow shade and alkalies produce a bluish shade.  $\text{Na}_2\text{S}_2\text{O}_4$  reduces orchil, but the color is restored by air oxidation (differing from cochineal). The characteristic property of orchil is to dye, strip, and redye wool readily.

(f) *Caramel*.—A number of tests have been developed for this coloring matter, most of them being based upon the insolubility in ether,  $\text{CHCl}_3$ , or amyl alcohol. Probably the most sensitive test is the Woodman-Newhall<sup>23</sup> modification of Amthor's test with a slight deviation. To 10–20 cc of a neutral soln of the color in a small centrifuge tube add 2 cc of 5%  $\text{ZnCl}_2$  and 2 cc of 2% KOH soln, stir well, and centrifuge. Pour off the liquid, and to the magma add 25 cc of boiling  $\text{H}_2\text{O}$ . Mix, centrifuge, and pour off liquid. Repeat this operation until the aqueous wash liquor is colorless. Dissolve the precipitate with 15 cc of 10% acetic acid, concentrate, neutralize carefully, and filter. Divide into 2 portions. To one add 3–5 volumes of paraldehyde in a 50 cc glass-stoppered cylinder, and just sufficient absolute alcohol to form a homogeneous soln (avoid excess). Caramel will be indicated by the formation of a brownish precipitate on standing. To the other portion of the caramel soln add an equal volume of a freshly prepared reagent consisting of phenylhydrazin hydrochloride, 2 parts; Na acetate, 3 parts;  $\text{H}_2\text{O}$ , 20 parts. A dark brown precipitate is formed in the presence of caramel.

## COMMERCIAL COAL TAR FOOD COLORS<sup>24</sup>

17

### PREPARATION OF SAMPLE

Thoroughly mix and without interruption weigh out the portions required for the determinations to be made. If the weighing cannot be made directly into the dish in which the determination is to be made, use weighing bottles for this purpose, placing in each a quantity approximating the weight called for, and weigh immediately.

18

### MOISTURE

(a) *Amaranth, ponceau 3R, ponceau SX, erythrosine, orange I, naphthol yellow S, sunset yellow FCF, tartrazine, guinea green B, light green SF yellowish, fast green FCF, brilliant blue FCF, and indigotine*.—Weigh approximately 2 g of the sample in a weighed Al dish 2 in. in diameter or in a weighing bottle of about the same diameter, dry in an air oven at  $135^\circ$  for 6 hours or overnight, cool over  $\text{H}_2\text{SO}_4$  in a desiccator, and weigh. Heat again for 1 hour, cool in the desiccator, and weigh. Repeat the heating and weighing at hour intervals until the weight becomes constant. Report the loss in weight as moisture.

(b) *For yellow AB and yellow OB*.—Proceed as directed under (a), heating the dye to  $80^\circ$  instead of to  $135^\circ$ .

### WATER-INSOLUBLE MATTER

19

### APPARATUS

*Prepared Gooch crucible*.—Digest a good grade of retentive asbestos with HCl (1+3), wash free from acid, and elutriate to remove fine particles; pour a sufficient quantity into a Gooch crucible placed in a filter flask to make a mat  $\frac{1}{2}$  in. thick

when packed. Using gentle suction, pack the asbestos down evenly with a tamping rod and then remove the Gooch from the filter flask. Loosen the mat around the edges with a thin narrow blade or pin, take out of Gooch, replace in an inverted position, pack down tightly, and add more of the asbestos suspension until a well packed mat of suitable thickness is obtained. Wash with hot  $H_2O$ , dry, ignite, rewash, dry at  $100-105^\circ$ , cool in a desiccator, and weigh. Repeat the washing, heating, and drying until constant weight is obtained.

## 20

## DETERMINATION

(a) *Amaranth, ponceau SX, erythrosine, naphthol yellow S, sunset yellow FCF, tartrazine, guinea green B, light green SF yellowish, fast green FCF, and brilliant blue FCF.*—Dissolve 5 g of dye in 200 cc of hot  $H_2O$  and allow the soln to cool to room temp. Filter thru the prepared Gooch crucible, wash with cold  $H_2O$  until all dissolved dye has been removed, dry at  $135^\circ$ , cool in a desiccator, and weigh. Report the increase in weight as total insoluble matter.

(b) *Ponceau 3R, orange I, and indigotine.*—Dissolve 5 g of dye in hot  $H_2O$ , using 250 cc for ponceau 3R and orange I, and 500 cc for indigotine, and boil, with frequent stirring, for 3 min. Cool the soln to room temp., let stand overnight and filter with moderate suction. Wash with cold  $H_2O$ , dry, cool, and weigh as directed under (a).

## 21

## NON-VOLATILE WATER-INSOLUBLE MATTER

*Amaranth, ponceau 3R, ponceau SX, erythrosine, orange I, naphthol yellow S, sunset yellow FCF, tartrazine, guinea green B, light green SF yellowish, fast green FCF, brilliant blue FCF, and indigotine.*—Incinerate the Gooch containing the total insoluble matter, 20, at a low red heat until all the organic matter has been volatilized. Cool in a desiccator and weigh.

## SODIUM CHLORIDE

## 22

## REAGENTS

All reagents must be halogen free.

*Sulfur dioxide soln.*—Saturate ice-cold distilled  $H_2O$  with  $SO_2$ . Keep the soln stoppered and in a cold place.

## 23

## DETERMINATION

(a) *Amaranth, ponceau 3R, ponceau SX, orange I, sunset yellow FCF, tartrazine, guinea green B, light green SF yellowish, fast green FCF, brilliant blue FCF, and indigotine.*—Thoroughly mix 5 g of dye with 4-6 g of  $K_2CO_3$  or  $Na_2CO_3$  in a 50 cc Pt or Ni crucible and moisten with  $H_2O$  or 50% alcohol. Cover evenly with about 1 g of the powdered carbonate, dry, and ignite at low red heat until organic matter is destroyed. Allow to cool and add enough  $H_2O$  to form a thin paste. If any lumps are present, break them up with a glass rod in order to produce a uniform suspension. Wash the mixture into a 250 cc volumetric flask with 100-150 cc of hot  $H_2O$  and allow to stand until all soluble salts are dissolved and the mixture is cold. Dilute to the mark with  $H_2O$ , mix thoroughly, and filter thru a dry paper.

Place a 200 cc portion of the filtrate in a 600 cc beaker and add enough of a 6-7% soln of  $KMnO_4$  to oxidize the sulfides and produce a permanent pink color. Add about 50 cc of  $H_2O$  and a slight excess of 10%  $AgNO_3$  soln (usually 6-8 cc is sufficient). Partially cover the beaker with a watch-glass and acidify the soln by care-

fully adding about 12 cc of  $\text{HNO}_3$ . Heat nearly to boiling, then add the saturated  $\text{SO}_2$  soln. Boil until any excess of  $\text{SO}_2$  is removed, cool, filter thru a weighed Gooch crucible, wash the precipitate of  $\text{AgCl}$  with hot  $\text{H}_2\text{O}$ , dry crucible and its contents at  $135^\circ$ , cool in a desiccator, and weigh. Calculate to percentage of  $\text{NaCl}$ .

(b) *Erythrosine*.—In a 500 cc volumetric flask dissolve 5 g of the dye in 400 cc of  $\text{H}_2\text{O}$ . Precipitate the color acid by adding a mixture of 2 cc of  $\text{HNO}_3$  and 10–20 cc of  $\text{H}_2\text{O}$ , dilute to 500 cc, mix, and filter thru a dry paper. Treat 200 cc of the filtrate with slightly more 10%  $\text{AgNO}_3$  soln than is required to precipitate the halogens present, add 5 cc of  $\text{HNO}_3$ , and heat to boiling. Cool, collect the precipitate in a weighed Gooch crucible, wash, dry, and weigh as directed under (a). If  $\text{NaI}$  is present, determine as directed under 54, and subtract the weight of  $\text{AgI}$  from the weight of the precipitate. Calculate the percentage of  $\text{NaCl}$  from the net  $\text{AgCl}$ .

(c) *Naphthol yellow S*.—Dissolve 5 g of the dye in 250 cc of  $\text{H}_2\text{O}$  and filter if necessary. Add 5 cc of  $\text{HNO}_3$  and precipitate the chloride by adding a slight excess of 10%  $\text{AgNO}_3$  soln. Boil for a few min., cool, and filter thru a weighed Gooch crucible. Wash, dry, and weigh the precipitate and calculate as directed under (a).

(d) *Ponceau 3R*.—Dissolve 5 g of the dye in 150 cc of hot  $\text{H}_2\text{O}$ , wash into a 250 cc volumetric flask, and add 25 cc of a 10% soln of  $\text{Ba}(\text{NO}_3)_2$ . Cool the mixture, make up to the mark, mix, and filter thru a dry paper. Acidify 100 cc of the filtrate (representing 2 g of dye) with  $\text{HNO}_3$  and treat with a slight excess of 10%  $\text{AgNO}_3$  soln. Collect the filtrate on a weighed Gooch crucible, wash, cool in a desiccator, weigh, and calculate as directed under (a).

## 24

## SODIUM SULFATE

(a) *Amaranth, ponceau 3R, ponceau SX, orange I, sunset yellow FCF, and indigotine*.—Transfer to a 250 cc volumetric flask that volume of a water soln of the sample which contains 5 g of the dye; add  $\text{H}_2\text{O}$ , if necessary, to bring the volume to 200 cc; and heat on the steam bath. Add pulverized  $\text{NaCl}$  as follows: For amaranth, tartrazine, and sunset yellow FCF, 70 g; for ponceau 3R, ponceau SX, orange I, indigotine, 50 g. Stopper the flask and shake at frequent intervals for an hour. (To hasten the precipitation the soln may be cooled in ice  $\text{H}_2\text{O}$ .) Dilute to the mark with saturated  $\text{NaCl}$  soln, shake, and filter on a dry 18 cm paper. To 100 cc of the filtrate add 200 cc of  $\text{H}_2\text{O}$  and 1 cc of  $\text{HCl}$  (1+9), heat to boiling, and add a slight excess of hot 10%  $\text{BaCl}_2$  soln. Allow to stand overnight, filter thru a weighed Gooch crucible, wash the precipitate of  $\text{BaSO}_4$  thoroly with hot  $\text{H}_2\text{O}$ , dry, ignite, cool in a desiccator, and weigh. Calculate the weight of  $\text{Na}_2\text{SO}_4$  equivalent to the  $\text{BaSO}_4$  obtained.

(b) *Erythrosine*.—To such a volume of a water soln of the sample as contains 1 g of dye add  $\text{H}_2\text{O}$ , if necessary, to bring the volume to 100 cc and then add 50 cc of  $\text{HNO}_3$  (1+49). Shake, and let settle. Filter off the dye and wash once, collecting the washings with the filtrate. Redissolve the dye by passing thru the filter paper as little  $\text{NH}_4\text{OH}$  (1+1) as will effect soln. Neutralize the base and reprecipitate the color acid by means of the  $\text{HNO}_3$  (1+49). Filter off the dye and wash once, collecting the filtrate and washings with those obtained after the first precipitation of color acid. [A 100 cc aliquot free of the color acid. 23(b), may be substituted.] Precipitate and determine the  $\text{BaSO}_4$  as directed under (a).

(c) *Light green SF yellowish, Guinea green B, fast green FCF, and brilliant blue FCF*.—Transfer to a 250 cc flask that volume of a soln which contains 5 g of the dye; add  $\text{H}_2\text{O}$ , if necessary, to bring to a volume of about 200 cc, and heat on the steam bath. Add 5 g of phosphotungstic acid and shake at intervals until dissolved.

Then add 50 g of pure pulverized NaCl, shaking at intervals to dissolve the salt. Cool, dilute to mark with saturated pure NaCl soln, shake, and filter. To 100 cc of the filtrate add 200 cc of H<sub>2</sub>O and 1 cc of HCl (1+9) and determine BaSO<sub>4</sub> as directed under (a).

(d) *Naphthol yellow S*.—To a volume of a water soln of the dye that contains 5 g of the sample in a 500 cc volumetric flask, add H<sub>2</sub>O, if necessary, to bring to a volume of about 300 cc. Add hot saturated KCl soln to bring to the 500 cc mark. Shake frequently until practically all the dye is precipitated. After the mixture is cold dilute with H<sub>2</sub>O to 500 cc. Shake, and filter thru a dry paper. Complete the determination as directed under (a) beginning with "To 100 cc of the filtrate add 200 cc of H<sub>2</sub>O and 1 cc of HCl (1+9)."

## 25

## SODIUM ACETATE

*Brilliant blue FCF*.—Weigh 10 g of the dye into a 200 cc Kjeldahl flask, add 25 cc of H<sub>2</sub>O, and connect the flask in an upright position to a vertical straight-tube water-jacketed condenser. Insert a separatory or dropping funnel thru the stopper of the flask, together with the tube leading from the flask to the condenser. Add 15 cc of phosphoric acid (sp. gr. 1.7) and heat the contents of the flask to boiling. Collect the acetic acid and condensed steam in a 300 cc Erlenmeyer flask to which has been added a standard soln of NaOH. Continue the boiling until about 250 cc has been distilled over, replacing the distillate by H<sub>2</sub>O from the dropping funnel so that the volume in the Kjeldahl flask remains about 20 cc. (This distillation should require about 2 hours.) At the end of the distillation period, remove the receiver and titrate the excess alkali with standard acid, using phenolphthalein as indicator. Run a blank distillation and deduct the resulting acidity found. From the corrected acidity calculate the quantity of Na acetate present in the dye.

## 26

## SULFATED ASH

(a) *Amaranth, ponceau 3R, ponceau SX, erythrosine, orange I, naphthol yellow S, sunset yellow FCF, tartrazine, guinea green B, light green SF yellowish, fast green FCF, brilliant blue FCF, and indigotine*.—Weigh accurately in a weighing bottle about 5 g of the dye and transfer it to a Pyrex Kjeldahl flask or tall beaker, washing out the weighing bottle with a little H<sub>2</sub>O. Destroy the organic matter to a convenient extent by digestion, using 15 cc of H<sub>2</sub>SO<sub>4</sub> and adding HNO<sub>3</sub> as required. As the bulk of the HNO<sub>3</sub> is driven off, lower the flame to avoid reaction on the glass. Transfer the mixture to a weighed Pt dish and heat over a ring burner, using at first a low flame at a safe distance below the dish, increasing the flame, and bringing it closer to the dish by gradual steps. Thus continue the destruction of the organic matter and the volatilization of the acids. Continue the heating until the production of acid fumes decreases. If C remains, remove the flame; let the mass cool a little; and add H<sub>2</sub>SO<sub>4</sub> dropwise, until the mass is moistened. Repeat the treatment until the C is burned off and the ash is white or reddish. Heat carefully with a blast lamp until fusion takes place with the production of a clear liquid free from bubbles. Cool in a desiccator and weigh. After deducting the weight of Na<sub>2</sub>SO<sub>4</sub> equivalent to the inorganic Na salts (chlorides, sulfates, carbonates, etc.) found in the other determinations, calculate to percentage of metallic Na combined in the dye.

(b) *Yellow AB and yellow OB*.—In a weighed Pt dish weigh 5 g of the dye, heat at a low temp. until most of the dye has been volatilized, moisten the residue with H<sub>2</sub>SO<sub>4</sub>, and complete the determination as directed under (a), beginning with "Repeat the treatment until the C is burned off."

## 27

## HEAVY METALS

(a) *Amaranth, ponceau 3R, ponceau SX, erythrosine, orange I, naphthol yellow S, sunset yellow FCF, tartrazine, guinea green B, light green SF yellowish, fast green FCF, brilliant blue FCF, and indigotine.*—Moisten the sulfated ash obtained under 26 with a few cc of HCl and evaporate to dryness on the steam bath. Warm the residue with 20 cc of HCl (1+19) until all soluble material has dissolved, transfer to a 100 cc volumetric flask, dilute to 100 cc, mix, and filter thru a dry paper. Reserve two 40 cc aliquots for the determination of Al, Ca, Fe, and Mg. Pour 20 cc of the filtrate into a test tube and pass in a washed stream of  $H_2S$  for 30 min. No turbidity other than that due to precipitated S should appear. If a colored precipitate is formed, filter and test it for Cu and Sn.

(b) *Yellow AB and yellow OB.*—The color of the sulfated ash obtained under 26 shows whether it is mainly  $Fe_2O_3$  or  $SiO_2$ . In either case fuse it with 1 g of  $K_2CO_3$  until the silicates have been decomposed. Moisten the residue with 2 or 3 cc of HCl, evaporate to dryness on the steam bath, and treat as directed under (a). If only a trace of  $Fe_2O_3$  or  $SiO_2$  is present in the original ash, the fusion with  $K_2CO_3$  may be omitted.

## 28

LEAD<sup>28</sup>

(Applicable to all permitted dyes.)

Place 5 g of the dye in a tall form 500 cc Pyrex beaker, cover with a watch-glass, add 15 cc of  $HNO_3$ , and let boil, or heat gently till the rapid evolution of brown fumes has ceased. Add 15 cc of  $H_2SO_4$  and continue the heating. Add small quantities (1–2 cc) of  $HNO_3$  at intervals until the organic matter is destroyed and the soln is colorless or at most a pale yellow. Continue the heating, with the evolution of dense white fumes, until a very small quantity (3–5 cc) of soln remains in the beaker. Cool the soln, which should form white or pale yellow crystals, and add 15–20 cc of  $H_2O$ . Re-evaporate the soln thus formed to white fumes, cool, take up in 100 cc of  $H_2O$ , add 100 cc of 95% ethyl alcohol, and let stand overnight. Filter out the precipitate of  $PbSO_4$ , which may be present in such small quantity as to escape detection with the naked eye, and wash thoroly with 50% ethyl alcohol (about 100 cc). Two 9 cm C. S. & S. No. 590 filter papers, or a suitable fritted glass crucible, have been found satisfactory for retaining the  $PbSO_4$ .

Place the filter paper in a small beaker, add 20 cc of 40%  $NH_4$  acetate, and heat to boiling, breaking up the paper with a glass rod. Filter thru an S. & S. No. 590 9 cm paper, or thru a fritted glass crucible, into a 100 cc colorimeter tube and wash with 10%  $NH_4$  acetate soln until the 50 cc mark is reached. If desired, filter into a 50 cc volumetric flask and take an aliquot portion to be used in the colorimeter tube. Prepare standards containing known quantities of Pb for comparison. To these add the same quantity of  $NH_4$  acetate as was used with the sample and dilute all tubes to a definite volume with  $H_2O$ . To each tube add 2 or 3 drops of glacial acetic acid and 10 cc of freshly prepared  $H_2S$  water. Shake the tubes to insure thoro mixing and estimate the quantity of Pb by comparison with the standards. Run blanks on all reagents used.

## 29

IRON, ALUMINUM, CALCIUM, AND MAGNESIUM<sup>29</sup>

(Applicable to all permitted dyes.)

To one of the two portions reserved under 27, add 5 g of  $NH_4Cl$  and neutralize with  $NH_4OH$  (1+1), boiling to drive off any excess. If the precipitate is very slight,



it may be disregarded; otherwise, filter thru a quantitative paper, wash with  $H_2O$  containing a trace of  $NH_4OH$  (reserving the filtrate and washings), and ignite paper and precipitate in a weighed crucible. Weigh the mixture of  $Fe_2O_3$  and  $Al_2O_3$ . Place the mixed oxides in a 500 cc Erlenmeyer flask and dissolve in aqua regia, boiling to drive off  $Cl$ . Add  $H_2O$  to bring the volume to about 75 cc and add  $NH_4OH$  to incipient precipitation. Dissolve the precipitate with as little  $HCl$  as possible, cool, and titrate the ferric iron present with 0.1  $N$   $TiCl_3$  soln, 37, using 5 g of  $NH_4CNS$  as indicator. Calculate the  $Fe$  as  $Fe_2O_3$ . To calculate the quantity of  $Al_2O_3$ , deduct the weight of ferric oxide from the total weight of mixed oxides. From the weight of the oxide calculate the percentage of metallic  $Al$ . Pass a washed stream of  $H_2S$  into the alkaline filtrate from the  $Fe$ - and  $Al$ -hydroxides. A white precipitate indicates the presence of  $Zn$ .

To the other reserved portion add 250 cc of  $H_2O$  to insure a low concentration of  $Mg$ , if present. Heat to boiling and add 3.5 g of  $NH_4Cl$  and enough  $NH_4OH$  soln (1+99) to make the soln barely alkaline. Filter off the precipitated hydroxides of  $Fe$  and  $Al$ . Wash and discard the precipitate. Heat the combined filtrate and washings to boiling and add 1 g of  $NH_4$  oxalate. After cooling and letting stand for an hour, filter thru an asbestos mat prepared on a small Witt plate in a glass funnel and wash with very little  $H_2O$ , reserving the combined filtrate and washings. Place the mat in a beaker, add 100 cc of  $H_2O$  and 2 cc of  $H_2SO_4$ , heat gently until the  $Ca$  oxalate dissolves, and titrate with 0.1  $N$   $KMnO_4$  soln. Calculate as metallic  $Ca$ .

Heat to boiling the reserved filtrate and washings and add a  $N$  soln of  $NaNH_4HPO_4$  until there is no further precipitation. While stirring add about  $\frac{1}{2}$  the volume of  $NH_4OH$  (1+9). Let stand 3 hours, filter thru an ashless paper, and wash with  $NH_4OH$  (1+49). Ignite the filter and precipitate in a weighed crucible, cool in a desiccator, and weigh the  $Mg_2P_2O_7$ . Calculate as metallic  $Mg$ .

#### ARSENIC

##### 1. By Direct Precipitation

#### 30

##### REAGENTS

(a) *Ammonium hydroxide*.—As-free and containing not less than 26% by weight of  $NH_3$ .

(b) *Sodium phosphate soln*.—As-free and containing 100 g of the crystallized salt per liter, or an equivalent quantity of  $H_3PO_4$ .

(c) *Magnesia mixture*.—As-free and containing 55 g of hydrated  $MgCl_2$ , 55 g of  $NH_4Cl$ , and 88 cc of  $NH_4OH$  per liter.

The other reagents and solns used are described under XXIX, 1, and the apparatus is described under XXIX, 2.

#### 31

##### DETERMINATION

(a) *Amaranth, ponceau SX, naphthol yellow S, sunset yellow FCF, tartrazine, guinea green B, light green SF yellowish, fast green FCF, and brilliant blue FCF*.—Dissolve 10 g of the dye in 250 cc of  $H_2O$  and add 10 cc of strong  $Br$  water. Make the mixture alkaline with 1–2 cc of the  $NH_4OH$ ; then add 20 cc of the  $Na$  phosphate soln and a slight excess of the  $Mg$  mixture. (The quantity of  $Mg$  mixture used must be from 1–5 cc in excess of that required to precipitate the phosphate completely, as ascertained previously by experiment.) Add the  $Mg$  mixture to the dye mixture slowly, stirring the soln during the addition. Add 10 cc of the  $NH_4OH$  and allow the mixture to stand for at least 30 min. Filter thru an 18 cm paper and wash with

NH<sub>4</sub>OH (1+9) until practically all dye is removed; then wash with about 5 cc of H<sub>2</sub>O. Allow the filter containing the washed precipitate to drain for 15–30 min. to remove most of the adhering liquid. Finally dissolve the Mg-NH<sub>4</sub> phosphate and arsenate by pouring 40 cc of arsenic-free HCl (1+3) over the filter in small portions and letting it drain into the generator bottle. Complete the determination as directed under XXIX, 4, beginning with, "add 5 cc of the KI reagent."

(b) *Erythrosine*.—Dissolve 18 g of the dye in 425 cc of H<sub>2</sub>O and add 5 cc of Br water and 20 cc of arsenic-free HCl (1+3). Mix, filter, and treat 250 cc of the filtrate (corresponding to 10 g of dye) with the NH<sub>4</sub>OH soln, using a quantity sufficient to render it slightly alkaline (about 5 cc). Complete the determination as directed under (a), beginning with "then add 20 cc of the Na phosphate soln."

## 32

## II. After Treatment with Nitric Acid

(a) *Amaranth, ponceau 3R, ponceau SX, erythrosine, orange I, naphthol yellow S, sunset yellow FCF, tartrazine, and indigotine*.—Place 12.5 g of the powdered dye in a 600 cc Pyrex beaker; add 25 cc of the HNO<sub>3</sub>; and, if the dye tends to form a clot or cake, stir the mixture thoroly. Boil for about 5 min., add 150–200 cc of H<sub>2</sub>O, and dilute the mixture to 250 cc in a volumetric flask. Pour the mixture back into the beaker, allow to stand for a few min., and filter thru an 18 cm paper. Treat 200 cc of the filtrate (corresponding to 10 g of dye) with the NH<sub>4</sub>OH, using a measured quantity sufficient to make the soln slightly alkaline (approximately 25 cc). Complete the determination as directed under 31(a), beginning with "then add 20 cc of the Na phosphate soln."

(b) *Yellow AB and yellow OB*.—In a 400 cc beaker mix thoroly 12.5 g of the powdered dye with 200 cc of HNO<sub>3</sub> (1+9), heat to boiling for 5–10 min., allow to cool, add 25 cc of the strong NH<sub>4</sub>OH, dilute to 250 cc in a volumetric flask, and filter. Determine the As in a 200 cc portion of the filtrate (corresponding to 10 g of dye) as directed under 31(a), beginning with "then add 20 cc of the Na phosphate soln."

## 33

## III. As Total Arsenic

(Applicable to all permitted dyes.)

Weigh 10 g of the dye into a 500 cc Kjeldahl flask or tall 500 cc beaker provided with a cover. Treat with 15 cc of the H<sub>2</sub>SO<sub>4</sub> and 25 cc of the HNO<sub>3</sub> and digest slowly under a hood until the HNO<sub>3</sub> has been decomposed or volatilized and the mixture turns dark. Cautiously add a few cc of HNO<sub>3</sub> to the hot mixture, which will again become light yellow or orange, and heat to charring. Repeat the HNO<sub>3</sub> treatment until the soln no longer shows a tendency to darken and remains yellow or colorless when evaporated until SO<sub>3</sub> fumes are evolved. Allow the completely digested mixture to cool, add 200 cc of H<sub>2</sub>O, and make slightly alkaline with NH<sub>4</sub>OH. Determine the As in the soln as directed under 31(a), beginning with "then add 20 cc of the Na phosphate soln." The comparatively large quantities of reagents necessary for the destruction of the organic matter will usually contain appreciable quantities of As, for which correction must be made by determinations on blanks.

## ETHER EXTRACTIVES

## 34

## REAGENTS

*Washed ether*.—Wash 1 liter of ether with 3 successive 150 cc portions of H<sub>2</sub>O immediately before using.

## 35

## DETERMINATION

(a) *Amaranth, ponceau 3R, ponceau SX, orange I, naphthol yellow S, sunset yellow FCF, tartrazine, guinea green B, light green SF yellowish, fast green FCF, brilliant blue FCF, and indigotine.*—Place in a separatory funnel that volume of a soln that contains 10 g of the dye and add  $H_2O$ , if necessary, to bring the volume to 200 cc, and, in the case of indigotine, to 500 cc. Extract with 2 successive 100 cc portions of the washed ether, shaking for 1 min. during each extraction. Remove the ether by decantation into a clean funnel and rinse the first funnel with 5 cc of ether, decanting into the second funnel. Reserve the color soln. Wash the combined extracts with 20 cc portions of  $H_2O$  until the washings are colorless. Decant the ether into a beaker, rinse the funnel with 5 cc of ether, and decant into the same beaker. Place the beaker in a dust-free atmosphere, allow the ether to evaporate to a volume of 50 cc, and transfer to a weighed flat-bottomed 100 cc dish, previously dried to constant weight over  $H_2SO_4$  in a desiccator. Rinse the beaker with 5 cc of ether and drain into the same dish. Let the remainder of the ether evaporate and dry over  $H_2SO_4$  to constant weight. The result represents the neutral extract.

To the reserved color soln, add 2 cc of a 10% NaOH soln and extract and rinse with ether. Reserve the color soln. Wash the combined ether extracts and rinsings with 20 cc portions of 0.1 N NaOH soln (1+99) until the washings are colorless. Evaporate the ether, dry, and weigh. The result represents the alkaline extract.

To the color soln reserved from the alkaline extraction, add twice the volume of HCl (1+3) necessary to neutralize. Repeat the previous procedure, but do not reserve the color soln. Wash the ether extract with 0.1 N HCl (1+99) until the washings are colorless. The result represents the acid extract.

(b) *Erythrosine.*—Determine as directed under (a), omitting the acid extraction. In case of the neutral extraction, wash the combined ether extracts with three 20 cc portions of  $H_2O$ .

## 36

## SULFUR

*Amaranth, ponceau 3R, ponceau SX, orange I, naphthol yellow S, sunset yellow FCF, tartrazine, guinea green B, light green SF yellowish, fast green FCF, brilliant blue FCF, and indigotine.*—Place about 0.2 g of the sample in a Parr calorimetric bomb and mix thoroly with approximately 10 g of  $Na_2O_2$ . Add a few mg of S-free sugar if necessary to aid in igniting the mass. Close the bomb and ignite. When cool, open the bomb, place it in a 600 cc beaker, and cover the beaker with a watch-glass. Dissolve the residue by adding warm  $H_2O$  thru the lip of the beaker until the bomb is covered. Acidify the soln cautiously with HCl and filter if necessary. Determine the  $BaSO_4$  as directed under 24(a), beginning with "heat to boiling, and add a slight excess of hot 10%  $BaCl_2$  soln." Deduct the S equivalent to the  $Na_2SO_4$  determined under 24.

## COLOR ACID AND DYE

## I. By Titration with Titanium Trichloride

## 37

## REAGENT

*Standard titanium trichloride soln.*—To 200 cc of the commercial 15% soln of  $TiCl_3$ , add 150 cc of HCl and dilute to 2 liters. Make the soln approximately 0.1 N, place in a container with a H atmosphere provision,<sup>27</sup> and allow to stand for 2 days for absorption of residual O.

## 38

## STANDARDIZATION OF SOLUTION

*Method I.*—Prepare a liter of 0.1  $N$   $Fe_2(SO_4)_3$  by dissolving ingot iron, Bureau of Standards Sample 55, in 30 cc of  $H_2SO_4$ . Dilute to about 400 cc, adding slowly, with stirring, a soln of pure  $KMnO_4$  (3.16 g dissolved in about 200 cc  $H_2O$ ) until a faint but perceptible reddish tint results. The last few cc should be added dropwise. Cool and dilute to 1 liter. Measure 20 cc of the 0.1  $N$   $Fe_2(SO_4)_3$  into a 500 cc flask, pass in a strong stream of  $CO_2$ , and add the  $TiCl_3$  rapidly until near the end point. Add 5 g of pure  $NH_4CNS$  and resume the addition of  $TiCl_3$  carefully until the red color just disappears.

*Method II.*—Make up a 0.1  $N$  soln of  $KMnO_4$  and standardize carefully, using Na oxalate, Bureau of Standards Sample 40, according to the directions supplied with the sample. Weigh 3 g of  $FeSO_4(NH_4)_2SO_4 \cdot 6H_2O$  and transfer to a 500 cc flask. Introduce a stream of  $CO_2$  and add 50 cc of recently boiled  $H_2O$  and 25 cc of 40% (by weight)  $H_2SO_4$ . Then, without interrupting the current of  $CO_2$ , add rapidly 40 cc of the standardized  $KMnO_4$ . Add  $TiCl_3$  until near the calculated end point. Then add quickly 5 g of  $NH_4CNS$ , and complete the titration. Run a blank on 3 g of  $FeSO_4(NH_4)_2SO_4 \cdot 6H_2O$ , using the same quantities of  $H_2O$ , acid,  $NH_4CNS$ , and the current of  $CO_2$ .

## 39

## INDICATOR

For many dyes the  $TiCl_3$  titration end point is indicated by a sharp decolorization. For some dyes the change is so gradual that an excess of  $TiCl_3$  (not more than 0.3 cc of approximately 0.1  $N$  soln) is required, and a suitable standard soln of some other dye must be used for the back titration, methylene blue serving well for this purpose. In other cases it is better to use an indicator which is reduced after the original dye has reacted with the  $TiCl_3$ . Thus a known quantity of light green SF yellowish serves well for this purpose.

Prepare a dye soln of such strength that 50 cc will require approximately 20 cc of the standard  $TiCl_3$  soln for its reduction. See Table 3.

## 40

## DETERMINATION

(a) *Amaranth, ponceau 3R, and sunset yellow FCF.*—Place in a 500 cc Erlenmeyer flask a volume of soln that corresponds to 20 cc of 0.1  $N$   $TiCl_3$ . Add 10 g of Na citrate and  $H_2O$  if necessary to bring the volume to 150 cc. Introduce a stream of  $CO_2$  and titrate with the standardized  $TiCl_3$ , keeping the  $CO_2$  flow continuous to the end.

(b) *Orange I, ponceau SX, tartrazine, guinea green B, light green SF yellowish, fast green FCF, brilliant blue FCF, and indigotine.*—Proceed as directed under (a),

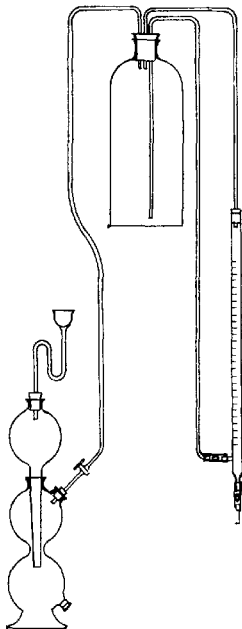


FIG. 22.—APPARATUS FOR TITRATION WITH TITANIUM TRICHLORIDE

substituting 15 g of Na acid tartrate for Na citrate and bringing the volume to 150 cc.

(c) *Naphthol yellow S*.—Proceed as directed under (b), using as indicator that volume of light green SF yellowish standardized soln (freshly made) that contains 10 mg of dye. Run a blank on the tartrate, light green SF yellowish, and  $\text{H}_2\text{O}$ .

TABLE 3.—Quantities of color acids and of pure coal-tar dye equivalent to 1 cc of 0.1 *N* titanium trichloride solution

DYE	MOLECULAR WEIGHT OF COLOR ACID	COLOR ACID EQUIVALENT TO 1 CC 0.1 <i>N</i> $\text{TiCl}_3$	DYE EQUIVALENT TO 1 CC 0.1 <i>N</i> $\text{TiCl}_3$
Amaranth.....	538.4	0.01346	0.01511
Ponceau 3R.....	450.4	.01126	.01236
Ponceau SX.....	436.3	.010907	.012006
Orange I.....	328.3	.008207	.008756
Naphthol yellow S.....	314.2	.002618	.002985
Sunset yellow FCF.....	408.3	.010206	.011305
Tartrazine.....	468.3	.01171	.01336
Guinea green B.....	668.5	.03342	.03453
Light green SF yellowish.....	748.7	.03743	.03963
Fast green FCF.....	764.5	.03822	.040423
Brilliant blue FCF.....	748.5	.03743	.03963
Indigotine.....	422.3	.02112	.02332

## 41

*II. By Precipitation*<sup>28</sup>

*Erythrosine*.—To that volume of  $\text{H}_2\text{O}$  soln of the sample that contains 0.25 g of dye, add, if necessary, sufficient  $\text{H}_2\text{O}$  to bring the volume to 100 cc. Then add 5 cc of  $\text{HNO}_3$  of approximately 0.6 *N* strength and filter thru a weighed Gooch crucible. Wash thoroly with 0.5%  $\text{HNO}_3$  and finally with not more than 10 cc of  $\text{H}_2\text{O}$ . Do not allow the precipitate to cake in the crucible until the washing has been completed. Dry to constant weight at 135°.

## PURE COAL TAR DYE

42 *I. By Direct Titration with Standard Titanium Trichloride Solution*

*Yellow AB and yellow OB*.—Dissolve 15 g of Na acid tartrate in 100 cc of  $\text{H}_2\text{O}$  and add 0.1 g of dye dissolved in 100 cc of 95% alcohol. Titrate with the standard  $\text{TiCl}_3$  soln, 37, under  $\text{CO}_2$ , using as an indicator 10 mg of light green SF yellowish from a fresh standardized soln, as directed under 40(c). Run a blank as directed under 40(c), including also the 100 cc of 95% alcohol. 1 cc of 0.1 *N*  $\text{TiCl}_3$  = 0.006180 g of yellow AB and 0.006530 g of yellow OB. Calculate the percentage of pure dye.

## 43

*II. By Precipitation*

*Erythrosine*.—Multiply the percentage of color acid obtained under 41 by the factor 1.074.

## 44

*III. By Titration with Potassium Permanganate*

*Indigotine*.—Take that volume of a freshly made  $\text{H}_2\text{O}$  soln of the dye that contains 0.04 g of dye and dilute, if necessary, to 400 cc. Add 2 cc of  $\text{H}_2\text{SO}_4$  and titrate against approximately 0.02 *N* standard  $\text{KMnO}_4$  soln. The end point is shown by the production of a clear yellow color. The titer of the standard soln must be fixed by titration against a freshly made soln of indigotine of known purity, and the same conditions of concentration and acidity must be maintained.

45

## MATTER INSOLUBLE IN CARBON TETRACHLORIDE

*Yellow AB and yellow OB.*—In a 100 cc beaker mix 5 g of the dye with 50 cc of  $\text{CCl}_4$ , stir, and heat to boiling. Wash a Gooch crucible prepared as directed under 19 with  $\text{CCl}_4$  and heat at  $100\text{--}105^\circ$  to constant weight. Filter the hot dye soln thru the crucible, transferring to it the residue in the beaker, and wash with five 10 cc portions of  $\text{CCl}_4$ . Dry at  $100\text{--}115^\circ$  and weigh.

46

## WATER EXTRACTIVES AND DYE INTERMEDIATES

*Yellow AB and yellow OB.*—Place 10 g of the well-powdered dye in a 500 cc separatory funnel, add 100 cc of benzene, stopper, and mix until dissolved. Extract with two 100 cc portions of  $\text{H}_2\text{O}$ , and evaporate 100 cc of the extract in a weighed Pt or crystallizing dish on the steam bath. Dry in an oven at  $100\text{--}105^\circ$ , cool, and weigh. The result represents the neutral extractive. Test small portions of the remainder of the filtrate for chlorides, sulfates, and nitrates. If more than traces are present, make the proper analyses on aliquot portions of the filtrate.

## MELTING POINT

*Yellow AB and yellow OB.*—

47

## APPARATUS

The apparatus, Fig. 23, consists of a tube about 15 cm long and 3.5 cm in internal diameter, with a bulb of 5 cm internal diameter. Fill with glycerol to about the height indicated. Fit the tube with a cork stopper carrying a glass tube (A), 5 mm in diameter, which reaches nearly to the bottom of the bath; an ordinary test tube (B) in which a thermometer (C) is suspended by means of a rubber stopper in such a manner that the Hg column is wholly within the tube and the Hg bulb equidistant from its walls; a long stemmed thermometer (D), supported so as to reach a short distance below the tube (B); and an outlet tube (E) to permit the escape of air and vapor.

48

## DETERMINATION

To a capillary tube of 1 mm or smaller internal diameter, sealed at one end, transfer a small portion of the sample by inserting into the sample the open end of the capillary, removing, inverting, and gently tapping until the well packed substance fills the bottom of the tube to a height of 2–4 mm. Attach the capillary tube to the thermometer (C) by means of a small rubber band, so that the sample is placed at about the middle of the Hg bulb. Replace the thermometer in the tube, connect the tube A to the air blast, and force a fairly rapid stream of air bubbles thru the bath. Raise the temp. of the bath rapidly to within  $5^\circ$  of the approximate melting point of the sample.

Keep the temp. constant until the thermometer reading is within  $1^\circ$  of that of the bath. Then raise the temp. slowly until the melting point is observed. On approaching within  $0.5^\circ$  of the melting point, the substance darkens; the true melting point is indicated by the formation of a meniscus on the upper surface. When this con-

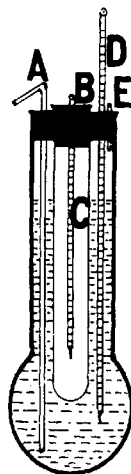


FIG. 23.—APPARATUS FOR DETERMINATION OF MELTING POINT

dition is observed, hold the temp. as nearly constant as possible until the whole of the sample has liquefied.

#### LOWER SULFONATED DYES

49

##### REAGENT

*Salt acetate soln.*—Dissolve 125 g of NaCl in  $H_2O$ , add 12 cc of glacial acetic acid and a soln of 13.6 g of Na acetate, and dilute to 500 cc.

50

##### PREPARATION OF SOLUTION

(a) *Amaranth, ponceau 3R, ponceau SX, sunset yellow FCF, tartrazine, and indigotine.*—Prepare a water soln of such strength that 50 cc will contain 0.2 g of the dye.

(b) *Light green SF yellowish, fast green FCF, and brilliant blue FCF.*—Prepare a water soln of such strength that 10 cc will contain 0.1 g of the dye.

51

##### DETERMINATION

(a) *Amaranth and tartrazine.*—To 50 cc of the prepared soln, 50(a), add 1 cc of HCl. Extract the lower sulfonated dye by shaking the soln successively in 3 separatory funnels, each containing 50 cc of amyl alcohol. Wash the amyl alcohol extracts by shaking successively with three 50 cc portions of 0.25 N HCl until the washings are practically colorless. Dilute the amyl alcohol in each funnel with 100 cc portions of gasoline (sp. gr. 0.65), and remove the lower sulfonated dye by washing with several 10 cc volumes of  $H_2O$ , passing each portion thru the 3 funnels in an order the reverse of that previously followed.

Determine the dye in the water extract by titration against standard  $TiCl_3$  soln, 37, using 15 g of Na acid tartrate, and having a volume of 100 cc. Run a blank determination on all reagents, using 1 mg of the dye concerned. Calculate the result to percentage of fast red E in amaranth and to fast yellow G in tartrazine. 1 cc of 0.1 N  $TiCl_3$  soln = 0.01256 g of fast red E and 0.01081 g of fast yellow G. If the quantity of dye is very low, it may be determined colorimetrically, amaranth or tartrazine, as appropriate, being used as standard.

(b) *Ponceau SX and sunset yellow FCF.*—Proceed as directed under (a), substituting 5% salt soln for 0.25 N HCl, and determine the quantity of dye colorimetrically by comparison with a standard soln of the dye.

(c) *Ponceau 3R.*—Proceed as directed under (a), substituting a mixture of equal volumes of amyl alcohol and gasoline (sp. gr. 0.65) in place of the amyl alcohol and running the blank with 1 mg of ponceau 3R. Calculate the result to percentage of Na trimethyl benzene-azo- $\beta$ -naphthol sulfonate, using the factor 0.009807.

(d) *Indigotine.*—Proceed as directed under (a), substituting 0.25 cc of acid for 1 cc, washing with 0.0625 N instead of 0.25 N acid, and running the blank with 1 mg of indigotine. Calculate the result to percentage of Na indigo monosulfonate, using the factor 0.01821.

(e) *Light green SF yellowish, fast green FCF, and brilliant blue FCF.*—To 10 cc of the prepared dye soln, 50(b), add 40 cc of the salt acetate soln and extract successively in 3 separatory funnels, each containing 100 cc of amyl alcohol. Wash the extracts with 100 cc portions of the salt acetate soln, passing each wash portion successively thru the 3 funnels in the order used for the original extractions. Remove the dye from the alcohol as directed under (a) and determine colorimetrically by comparison with a standard guinea green B soln of approximately the same strength for light green SF yellowish, by comparison with a standard soln of fast green FCF and brilliant blue FCF for subsidiary dyes in the latter. Report as percentage of guinea green B in light green SF yellowish and subsidiary dye in fast green FCF or brilliant blue FCF.

## 52

BOILING RANGE OF  $\Psi$  CUMIDINE FROM PONCEAU 3R

(a) Dissolve 60 g of the dye in a 600–700 cc beaker with about 450 cc of boiling  $H_2O$ , and add the hot soln very slowly to a warm (60–80°) soln of 100 g of  $SnCl_2$  in 100 cc of  $HCl$  in a tall liter beaker. Add the dye soln in 10–20 cc portions, waiting after each addition until the mixture is a pale brown; otherwise the dye will be precipitated, in which case it can be reduced only with difficulty. As reduction proceeds and the soln becomes more dilute, heat to boiling, taking care that the mixture does not boil over after each addition of dye, as some heat is generated by the reaction. After all the dye has been added and reduced, allow the mixture to cool, and make alkaline by the addition of about 75 g of  $NaOH$  dissolved in 150–200 cc of  $H_2O$ .

Cool the alkaline mixture and extract the cumidine by shaking it with three 200 cc portions of ether. Combine the ether extracts thus obtained and wash with  $H_2O$  until the alkali and salts are removed. Evaporate the solvent on the steam bath, but avoid such prolonged heating as may tend to volatilize the base. Transfer the residue of crude cumidine to a small side-necked flask and distil it, carefully avoiding overheating. Observe the range within which the substance volatilizes.

(b) Proceed as directed under (a) to the directions for the extraction with ether. Then steam distil the alkaline mixture until no more oil is carried over. Separate the oil layer and extract the  $H_2O$  layer with two 150 cc portions of ether. Add the extracts to the oil layer and wash the mixture with successive 10 cc portions of  $H_2O$  until the alkali and salts are removed. Evaporate the solvent and complete the determination as directed under (a).

## 53

## ISOMERIC AND SIMILAR DYES IN AMARANTH

Take a volume of an  $H_2O$  soln of the sample that contains 0.1 g of the dye and dilute, if necessary, to 40 cc with  $H_2O$ . Add 10 cc of 0.1  $N$  benzidine soln (9.2 g of base per liter in 0.5  $N$   $HCl$ ), mix well, and allow to stand exactly 2 min. Filter thru a fluted paper and dilute 10 cc of the filtrate to 100 cc. Compare this soln colorimetrically with a standard amaranth soln containing 0.4 mg of the dye per 100 cc. The soln of the amaranth to be tested may be used in making the standard soln. If, after the benzidine treatment, the soln obtained is not more intensely colored than the standard soln, the proportion of isomeric dyes may be considered to be below 1.5%.

## 54

## SODIUM IODIDE

*Erythrosine*.—Dilute to approximately 400 cc that volume of an  $H_2O$  soln of the sample that contains 5 g of the dye and add a mixture of 2 cc of  $HNO_3$  and 10–20 cc of  $H_2O$ . Dilute to exactly 500 cc, mix, and filter thru a dry paper. Place 200 cc of the filtrate in a porcelain casserole and make slightly alkaline with 10%  $NaOH$  soln. Add approximately 20 cc of 7%  $KMnO_4$  soln, mix, and add 10 cc of  $HNO_3$ . Place on a steam bath and evaporate to dryness. Add 5 cc of 7%  $KMnO_4$  and 5 cc of  $HNO_3$  and again evaporate to dryness. Then add approximately 50 cc of  $H_2O$ , 5 cc of  $HNO_3$ , and 25–30 cc of a saturated soln of  $SO_2$ . Stir frequently, breaking up any lumps, until the hydrated oxide of  $Mn$  has dissolved. Filter, wash the paper with  $H_2O$ , add to the combined filtrate and washings an excess of 10%  $AgNO_3$  soln, and boil until the  $SO_2$  has been expelled. Collect the precipitate on a weighed Gooch crucible, wash with hot  $H_2O$ , dry, and weigh. Calculate as percentage of  $NaI$ .

## 55

## IODINE ORGANICALLY COMBINED

*Erythrosine*.—Place in a porcelain casserole that volume of an  $H_2O$  soln of the



sample that contains 0.3–0.4 g of the dye. Add 5 cc of a 10% NaOH soln and 35 cc of a 7% soln of pure  $\text{KMnO}_4$ , and mix. Partially cover the vessel with a watch-glass and add 10 cc of  $\text{HNO}_3$ . Place on the steam bath and keep covered until spattering ceases; remove the watch-glass and allow evaporation to proceed to dryness, taking care to prevent access of reducing gases or vapors to the mixture. Treat the residue with 5 cc of 7%  $\text{KMnO}_4$  and 5 cc of  $\text{HNO}_3$  and again evaporate to dryness. Add approximately 50 cc of  $\text{H}_2\text{O}$ , 5 cc of  $\text{HNO}_3$ , and 40 cc of a saturated soln of  $\text{SO}_2$ , and let stand with occasional stirring (breaking up the lumps with a glass rod) until the hydrated oxide of Mn has dissolved.

Filter, wash the paper thoroly with  $\text{H}_2\text{O}$ , add an excess of 10%  $\text{AgNO}_3$  to the combined filtrate and washings, and boil until  $\text{SO}_2$  has been expelled and the AgI has flocculated. Collect the precipitate on a weighed Gooch crucible, wash with hot  $\text{H}_2\text{O}$ , dry, and weigh. Calculate as percentage of free I and from the result subtract the percentage of the I found as NaI, 54. This result is the I organically combined.

## 56

## TOTAL HALOGENS

*Erythrosine*.—Mix 0.5–1 g of the dye with 4 g of  $\text{K}_2\text{CO}_3$  and moisten to a paste with 50% alcohol. Dry, cover with a layer of dry  $\text{K}_2\text{CO}_3$ , and ignite at a low red heat. Allow to cool, moisten with a few drops of  $\text{H}_2\text{O}$ , and break up the charred mass thoroly. Wash into a beaker with about 20 cc of  $\text{H}_2\text{O}$ , allow to digest for 15 min., and filter. Wash the insoluble matter until the washings no longer react with  $\text{AgNO}_3$ ; then acidify the filtrate and washings with  $\text{HNO}_3$ , using an excess equivalent to 5 cc of the strong acid, and precipitate the halogens with 10%  $\text{AgNO}_3$  soln. Collect the precipitate on a weighed Gooch crucible, wash, dry, and weigh. Compare with the sum of the results obtained in the separate halogen determinations.

## 57

## SODIUM CARBONATE

*Erythrosine*.—Determine total  $\text{CO}_2$  as directed under XVII, 4, using a 10 g sample. Calculate and report as  $\text{Na}_2\text{CO}_3$ .

## 58

## ORANGE II IN ORANGE I

To a volume of an  $\text{H}_2\text{O}$  soln of the sample that contains 1 g of dye add  $\text{H}_2\text{O}$ , if necessary, to bring the volume to 100 cc, and 10 cc of  $\text{HCl}$ . Extract this soln by shaking successively in three 500 cc separatory funnels, each containing 100 cc of amyl alcohol and 5 cc of  $\text{HCl}$ . Wash each of the 3 amyl alcohol extracts by means of six 100 cc portions of  $N$   $\text{Na}_2\text{CO}_3$  soln (53 g of anhydrous  $\text{Na}_2\text{CO}_3$  to the liter), passed successively thru the funnels in the order first used. In washing the acidified amyl alcohol solns, shake gently at first, keeping the funnel upright and unstoppered until the evolution of  $\text{CO}_2$  is slow enough to permit more vigorous shaking. In the same manner wash the extracts in the second and third funnels with 2 more 100 cc portions of the  $\text{Na}_2\text{CO}_3$  soln and wash the extract in the third funnel with 2 additional portions of the carbonate soln. Dilute the amyl alcohol solns by adding 350 cc of gasoline (sp. gr. 0.65) to each funnel. Remove the dye by extracting completely with the requisite number of 10 cc portions of  $\text{H}_2\text{O}$  passed thru the funnels, reversing the order previously used. Bring the volume to 100 or 150 cc by adding  $\text{H}_2\text{O}$ ; add about 10 g of Na acid tartrate and titrate with standard  $\text{TiCl}_3$  soln, 37.1 cc of 0.1  $N$   $\text{TiCl}_3$  = 0.008756 g of orange II.

## 59

## MARTIUS YELLOW IN NAPHTHOL YELLOW S

Dissolve 5 g of the dye in 150 cc of  $\text{H}_2\text{O}$ , add 5 cc of  $\text{HCl}$ , and shake vigorously in a separatory funnel for 1 min. with 50 cc of gasoline (sp. gr. 0.65). Separate the solns and extract the aqueous liquid again with 25–30 cc of the solvent. Combine the

portions of gasoline, decant into a clean separatory funnel, and wash with four 25 cc portions of 0.25 *N* HCl. Remove the martius yellow by shaking with a few portions of 5% NaOH soln. Neutralize the alkaline dye soln with tartaric acid, add Na tartrate, if necessary, and titrate against standard  $\text{TiCl}_3$  soln as directed under 40(c). 1 cc of 0.1 *N*  $\text{TiCl}_3 = 0.002134$  g of martius yellow.

Very small quantities (less than 0.1%) may also be determined colorimetrically (in neutral or slightly alkaline soln) by comparison with a standard naphthol yellow S soln, the tinctorial power of which is considered to be 8/10 that of martius yellow.

#### TARTRAZINE AND AMARANTH<sup>20</sup>

60

##### REAGENTS

- (a) *Stannous chloride*.—40%. Add 40 g of  $\text{SnCl}_2$  to sufficient HCl to make 100 cc.
- (b) *Ammonia sodium chloride*.—To 12.5 g of NaCl and 20 cc of  $\text{NH}_4\text{OH}$  add sufficient  $\text{H}_2\text{O}$  to make 500 cc.
- (c) *Starch iodide paper*.—Triturate 10 parts of starch and 200 parts of  $\text{H}_2\text{O}$ , bring to a boil, and add 1 part of KI. Impregnate strips of white filter paper with this soln, dry, and preserve in glass-stoppered bottles.

61

##### DETERMINATION

Prepare a 1% soln of the mixed dyes. Determine the total color as directed in 40 by titrating definite volumes with 0.1 *N*  $\text{TiCl}_3$ , using sodium citrate as a buffer. A convenient charge (about 0.2 g of color) requires about 10 cc of the standard  $\text{TiCl}_3$ , 37. Make determinations in duplicate.

Pipet a definite volume (20 cc if product is pure color) of the dye soln into 250 cc centrifuge bottles and adjust to a volume of 50 cc with  $\text{H}_2\text{O}$ . Carefully add exactly 4 cc of the  $\text{SnCl}_2$  soln, mix well, and permit to stand, preferably overnight at room temp. (not below 20°). The following day place the bottles in a water bath previously heated to 50–60°, and maintain the contents of the bottles at that temp. for 5 min. (Since the reduction operation has an important bearing upon the results, the above directions must be observed closely.) Remove the bottles from the bath and permit to cool to room temp. (The reduced solns should be colorless or a very faint yellow.) Add exactly 5 cc of ammonia and mix with the contents, which should become slightly alkaline to litmus paper. Centrifuge, and decant thru a 15 cm quantitative filter into a 400 cc beaker surrounded by ice  $\text{H}_2\text{O}$ . Into the beaker measure 15 cc of HCl and 0.2 cc of 10%  $\text{CuSO}_4$  soln. Wash the residue in the bottles thrice with 50 cc portions of the ammonia sodium chloride soln, mix well, centrifuge each time, and decant thru the filter. (The total filtrate in the beaker should now measure about 200 cc.) Cool the contents of the beaker to 5° and add slowly 1 cc of 10%  $\text{NaNO}_2$  soln. Keep the temp. between 5 and 8° for 2 hours, testing with the starch iodide paper at intervals of about every 30 min. (There should be an excess of nitrous acid at the end of this period. Under ordinary conditions the quantity of  $\text{NaNO}_2$  stated is found sufficient.) Add 12 cc of 1% dilute alcoholic  $\beta$  naphthol soln to 100 cc of 2 *N*  $\text{Na}_2\text{CO}_3$  in a liter beaker and cool to 15°. Into the  $\beta$  naphthol soln pour gradually the diazo soln, stirring vigorously. Rinse the beaker with some of the dye soln and lastly rinse with 25 cc of alcohol and add to the dye soln. Heat the contents of the beaker over a steam bath, maintaining a temp. of about 70° for 1 hour, cool, and allow to stand overnight at room temp.

Use six separatory funnels of 250 cc capacity. Measure into each 50 cc of amyl alcohol. Add to the first funnel 50 cc of the alkaline dye soln. Shake vigorously and wait until a sharp separation has occurred; draw off the lower layer and pass suc-

cessively thru the other 5 funnels, and lastly discard the lower layer. Repeat the procedure with 50 cc portions until the entire dye soln is extracted. Rinse the beaker with 5-50 cc portions of 0.25 *N* HCl, passing them individually thru the amyl alcohol extracts, and later discarding. Dilute each amyl alcohol extract with 100 cc of petroleum ether and extract out the orange dye with 100 cc portions of 1/128 *N* HCl, shaking vigorously and passing the lower extracts thru the entire series of funnels. Collect the extracts containing the orange II in a liter casserole. Continue washing the amyl alcohol until all orange color is removed. (It will be noted that the first funnel will readily give up the orange dye inasmuch as the red is not readily washed out at that acid concentration.) Continue the acid extraction until each funnel will yield no more orange color.

In the presence of a large amount of red color, wash the last dilute acid extract with a mixture of amyl alcohol and petroleum ether (1:2) in order to remove any red dye which may have been extracted. Add an excess of ammonia (10 cc) to the casserole containing the orange color, and evaporate carefully to dryness on the steam bath, avoiding spattering. Dissolve the coloring matter from the casserole with four 25 cc portions of hot H<sub>2</sub>O and transfer to a 300 cc Erlenmeyer flask. Rinse the casserole with 10 cc of alcohol and add to flask; then add 10 g of sodium bitartrate and also 1 drop of 2% light green SF yellowish soln (indicator). Boil vigorously, pass in a rapid stream of CO<sub>2</sub>, and titrate with standard TiCl<sub>3</sub> soln, 37, until the green color is visible. Note reading and add another drop of the standard soln, whereupon the green color should be destroyed.

The number of cc of standard TiCl<sub>3</sub> solution required to reduce the orange II = the quantity of tartrazine originally present. 1 cc of 0.1 *N* TiCl<sub>3</sub> = 0.01336 g of tartrazine.

Subtract the above titration from the original total color titer. The difference is the volume necessary to reduce the amaranth originally present. 1 cc. of 0.1 *N* TiCl<sub>3</sub> = 0.01511 g of amaranth.

TABLE 4.—Percentage of sulfur, nitrogen and sodium in permitted food dyes

DYE	SULFUR	NITROGEN	SODIUM	SODIUM SULFATE CORRESPONDING TO SODIUM CON- TENT
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Amaranth.....	15.91	4.64	11.41	35.23
Ponceau 3R.....	12.97	5.66	9.30	28.71
Ponceau SX.....	13.35	5.83	9.57	29.55
Erythrosine.....	.....	.....	5.12	15.81
Orange I.....	9.15	8.00	6.57	20.28
Naphthol yellow S.....	8.95	7.82	12.84	39.64
Sunset yellow FCF.....	14.18	6.19	10.17	31.40
Tartrazine:				
Trisodium salt.....	12.00	10.49	12.91	39.85
Disodium salt.....	12.52	10.94	8.98	27.72
Guinea green B <sup>1</sup> .....	9.29	4.06	3.33	10.28
Light green SF yellowish <sup>1</sup> .....	.....	.....	.....	.....
Disodium salt.....	12.13	3.53	5.80	17.90
Monosodium salt.....	12.48	3.64	2.98	9.20
Fast green FCF.....	11.89	3.46	5.69	17.57
Brilliant blue FCF.....	12.13	3.53	5.80	17.91
Indigotine.....	13.75	6.01	9.86	30.42
Yellow AB.....	.....	17.00	.....	.....
Yellow OB.....	.....	16.09	.....	.....

<sup>1</sup> There is evidence that the disodium salt of guinea green B and the trisodium salt of light green SF yellowish form colorless solutions.

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## TOTAL NITROGEN

(a) *Guinea green B, light green SF yellowish, fast green FCF, brilliant blue FCF, and indigotine*.—Proceed as directed under II, 22, using 2 g of the sample and a little  $\text{CuSO}_4$  to assist the oxidation.

(b) *Amaranth, ponceau 3R, ponceau SX, orange I, sunset yellow FCF, tartrazine, Yellow AB, and yellow OB*.—Treat 2 g of the dye with 25 cc of a saturated soln of  $\text{SO}_2$  and 1 g of Zn dust, and warm the mixture gently until it becomes colorless (2–3 min.); if it does not become colorless, add more  $\text{SO}_2$  soln in small portions at a time until the color is destroyed. Then add 30 cc of  $\text{H}_2\text{SO}_4$  and 0.7 g of  $\text{HgO}$  or its equivalent of metallic Hg and digest the mixture. Finally make alkaline, distil, and titrate as directed under II, 19, 22, or 24.

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## XXII. DAIRY PRODUCTS

### MILK

#### 1 COLLECTION OF SAMPLE—OFFICIAL

The quantity of sample required depends upon the number of determinations to be made. For the usual analysis collect 250–500 cc ( $\frac{1}{2}$  pint) of sample; for the fat determination only, 50–60 cc (approximately 2 fl. oz.) will suffice.

In the case of bottled milk collect one or more bottles as prepared for sale. In sampling bulk milk thoroly mix by pouring from one clean vessel into another 3 or 4 times. If this procedure is impracticable, thoroly stir the milk for at least 30 seconds with a suitable appliance long enough to reach to the bottom of the container. If cream has formed on the milk, continue the mixing until all cream is detached from the sides of the vessel and evenly emulsified thruout the liquid.

Place the samples in non-absorbent, air-tight containers and keep them in the cold, but at a temp. above freezing, until ready for examination. When transported by mail, express, or otherwise, completely fill the containers, tightly stopper, and mark for identification. A suitable quantity of preservative ( $\text{HgCl}_2$ ,  $\text{K}_2\text{Cr}_2\text{O}_7$ , or  $\text{HCHO}$ ) may be used unless the presence of the preservative is objectionable in connection with physical or chemical tests to be applied in addition to the determination of fat.

#### 2 PREPARATION OF SAMPLE—OFFICIAL

Before withdrawing portions for analytical determinations, bring the sample to a temp. of 15–20° and mix thoroly by pouring into a clean receptacle and back until a homogeneous mixture is assured. If lumps of cream do not completely disappear, warm the sample to about 38°, mix thoroly, then cool to 15–20°. In case a measured volume is required in a determination, bring the temp. of the sample to 20° before pipetting.

#### 3 SPECIFIC GRAVITY—TENTATIVE

Determine specific gravity at 20/20° by means of a pycnometer, XIV, 3, or by means of a standardized hydrometer.

#### 4 ACIDITY—TENTATIVE

Dilute 10–20 cc of the milk with an equal volume of  $\text{CO}_2$ -free  $\text{H}_2\text{O}$  and titrate with standard  $\text{NaOH}$  soln, using phenolphthalein indicator. Express the result as percentage of lactic acid. The determination may be conveniently made by measuring 17.6 cc of the prepared sample with the 17.6 cc Babcock pipet, 20(b), diluting with an equal volume of  $\text{CO}_2$ -free  $\text{H}_2\text{O}$ , washing out the pipet with  $\text{CO}_2$ -free  $\text{H}_2\text{O}$ , and titrating with 0.1 N  $\text{NaOH}$  soln, using 0.5 cc of phenolphthalein indicator. Number of cc of 0.1 N  $\text{NaOH}$  soln required  $\div 20$  = the percentage of lactic acid.

#### CITRIC ACID—TENTATIVE

#### 5 PREPARATION OF SAMPLE

To 50 g of milk or 6 g of dry milk plus 44 cc of  $\text{H}_2\text{O}$  in a 150 cc beaker, add about 100 mg of tartaric acid and 6 cc of normal  $\text{H}_2\text{SO}_4$  and place on the steam bath for 15 min. Immediately add 3 cc of a 20% phosphotungstic acid soln, mix well, and return to the steam bath for 5 min. Transfer to a 250 cc volumetric flask with 95%

alcohol, cool, dilute to the mark with the alcohol, mix, and filter thru a folded paper. Pipet 200 cc of the clear filtrate into a centrifuge bottle.

6

## REAGENTS

Use the reagents specified under XXVI, 30.

7

## DETERMINATION

To the soln in the centrifuge bottle, add 10 cc of the Pb acetate soln, shake vigorously for about 2 min., and centrifuge at about 1000 r.p.m. for 15 min. Carefully decant the supernatant liquid from the precipitated Pb salts and test with a small quantity of the Pb soln. If a precipitate forms, return to the centrifuge bottle, add more Pb soln, shake, and again centrifuge. If the sediment lifts, repeat the centrifuging, increasing the speed and time. Allow the bottle to drain thoroly by inverting it for several min. To the Pb salts in the centrifuge bottle add about 150 cc of H<sub>2</sub>O, shake thoroly, and pass in H<sub>2</sub>S to saturation. Transfer to a 250 cc volumetric flask, dilute with H<sub>2</sub>O to mark, mix, and filter thru a folded paper.

From this point proceed as directed under XXVI, 31, beginning "Pipet 200 cc of the filtrate into a 500 cc Erlenmeyer flask and evaporate to 75 cc."

Calculate the mg of citric acid in the portion taken for analysis by the following formula:

$$X = \frac{1.05(0.424P + 0.017S)}{0.64}, \text{ or } 1.64(0.424P + 0.017S), \text{ in which}$$

$X$  = mg. of citric acid in the portion taken for analysis,

$P$  = weight of pentabromacetone in milligrams; and

$S$  = volume of filtrate (cc).

8

## TOTAL SOLIDS—OFFICIAL

Weigh a flat-bottomed dish of not less than 5 cm diameter. If desired, the dish may have spread in it, prior to weighing, 15–20 g of pure dry sand. Pipet into the dish 3–5 cc of the sample, weigh quickly, and heat at the temp. of boiling H<sub>2</sub>O until it ceases to lose weight. Cool in a desiccator and weigh quickly to avoid the absorption of moisture. Report the increase in weight as total solids.

9

## ASH—OFFICIAL

Into a weighed dish pipet about 20 cc of the prepared sample, weigh quickly, add 6 cc of HNO<sub>3</sub>, evaporate to dryness, and ignite at a temp. below redness until the ash is free from C. Cool in a desiccator, weigh, and report the increase in weight as ash.

10

## TOTAL NITROGEN—OFFICIAL

Transfer 5 g of the sample to a Kjeldahl digestion flask and proceed as directed under II, 21, 23, or 25. The percentage of  $N \times 6.38$  = percentage of N compounds.

## CASEIN

(This determination should be made while the milk is fresh, or nearly so. If it is not possible to make this determination within 24 hours, add 1 part of HCHO to 2500 parts of milk and keep in a cool place.)

11

## Method I.—Official

Place 10 g of the sample in a beaker with 90 cc of H<sub>2</sub>O at 40–42°, and add at once 1.5 cc of acetic acid (1+9). Stir, and let stand 3–5 min. Decant on a filter, wash by

decantation 2 or 3 times with cold  $H_2O$ , and transfer the precipitate to the filter. Wash once or twice on the filter. (The filtrate should be clear, or very nearly so.) If the first portions of the filtrate are not clear, repeat the filtration, and complete the washing of the precipitate. Determine N in the washed precipitate and filter paper as directed under II, 21, 23, or 25, and multiply by 6.38 to obtain the equivalent of casein.

To a sample of milk that has been preserved, the acetic acid should be added in small portions, a few drops at a time, with stirring, and the addition should be continued until the liquid above the precipitate becomes clear, or very nearly so.

#### *Method II.<sup>2</sup>—Tentative*

12

#### REAGENT

Pipet 250 cc of normal acetic acid into a 1000 cc flask. Add 125 cc of normal  $CO_2$ -free NaOH. Make up to 1000 cc with  $CO_2$ -free  $H_2O$  and mix thoroly.

13

#### DETERMINATION

Pipet 20 cc of the sample into a 100 cc flask. Add 50 cc of the reagent, mix, make up to volume with  $H_2O$ , and shake well. Set the flask in hot  $H_2O$  (50–60°, *not over 60°*) and let stand 15 min. Cool to room temp., add 0.5 g of celite analytical filter aid, shake thoroly, and filter clear thru a suitable folded paper, taking care to prevent evaporation during filtration. Determine N (A) in 50 cc of the clear filtrate, and determine total N (B) in 10 cc of the milk.  $(B - A) \times 6.38$  = the casein in 10 cc of the milk. Report grams of casein per 100 cc of milk, or divide the grams per 100 cc by the density of the milk and report as percentage by weight.

#### ALBUMIN

14

#### *Method I.—Official*

Exactly neutralize the filtrate obtained under 11 with 10% NaOH soln, add 0.3 cc of acetic acid (1+9), and heat on a steam bath until the albumin is completely precipitated. Collect the precipitate on a filter; wash with cold  $H_2O$ ; determine the N as directed under II, 21, 23, or 25, and multiply by 6.38 to obtain the equivalent of albumin.

#### LACTOSE

#### *Optical Method—Official*

15

#### REAGENTS

(a) *Acid mercuric nitrate soln.*—Dissolve Hg in twice its weight of  $HNO_3$  and dilute with an equal volume of  $H_2O$ .

(b) *Mercuric iodide soln.*<sup>3</sup>—Dissolve 33.2 g of KI and 13.5 g of  $HgCl_2$  in 200 cc of glacial acetic acid and 640 cc of  $H_2O$ .

16

#### DETERMINATION

Determine the specific gravity of the milk as directed under 3. The quantity of sample to be taken for the determination varies with the specific gravity and is to be measured at the same temp. at which the specific gravity is taken. The volume to be measured will be found in the table, 17, which is based upon twice the normal weight of lactose (32.9 g per 100 cc) for the Ventzke sugar scale.

Place the quantity of milk indicated under 17 in a flask graduated at 102.6 cc.

Add 1 cc of the acid  $\text{Hg}(\text{NO}_3)_2$  soln or 30 cc of the  $\text{HgI}_2$  soln (an excess of these reagents does no harm), fill to the mark, shake frequently for at least 15 min., filter thru a dry filter, and polarize. It is not necessary to heat before polarizing. If a 200 mm tube is used, divide the polariscope reading by 2 (or, if a 400 mm tube is used, by 4) to obtain the percentage of lactose in the sample.

17 *Volumes of milk corresponding to a lactose double normal weight<sup>4</sup>*

SPECIFIC GRAVITY OF MILK	VOLUME OF MILK FOR A LACTOSE DOUBLE NORMAL WEIGHT (VENTZKE SCALE)	SPECIFIC GRAVITY OF MILK	VOLUME OF MILK FOR A LACTOSE DOUBLE NORMAL WEIGHT (VENTZKE SCALE)
	cc		cc
1.024	64.25	1.030	63.90
1.025	64.20	1.031	63.80
1.026	64.15	1.032	63.75
1.027	64.05	1.033	63.70
1.028	64.00	1.034	63.65
1.029	63.95	1.035	63.55
		1.036	63.50

18 *Gravimetric Method—Official*

Dilute 25 g of the sample with 400 cc of  $\text{H}_2\text{O}$  in a 500 cc volumetric flask and add 10 cc of  $\text{CuSO}_4$  soln, **XXXIV**, 31(a), and about 7.5 cc of a  $\text{KOH}$  soln of such strength that 1 volume is just sufficient to precipitate completely the  $\text{Cu}$  as hydroxide from 1 volume of the  $\text{CuSO}_4$  soln. (Instead, 8.8 cc of 0.5 *N*  $\text{NaOH}$  soln may be used.) After the addition of the alkali soln, the mixture must still have an acid reaction and contain  $\text{Cu}$  in soln. Fill the flask to the 500 cc mark, mix, filter thru a dry filter, and determine lactose in an aliquot of the filtrate as directed under **XXXIV**, 57.

FAT

19 *Roege-Gottlieb Method<sup>5</sup>—Official*

Transfer 10 g of the sample to a Rörbrig tube or a similar apparatus, add 1.25 cc of  $\text{NH}_4\text{OH}$  (2 cc if the sample is sour) and mix thoroly. Add 10 cc of 95% alcohol and mix well. Add 25 cc of ether, shake vigorously for 30 seconds, add 25 cc of petroleum ether (redistilled slowly at a temp. below  $65^\circ$ ), and shake again for 30 seconds. Let stand 20 min., or until the upper liquid is practically clear. Draw off into a flask thru a small, quick-acting filter as much as possible of the ether-fat soln (usually 0.5–0.8 cc will be left). Again extract the liquid remaining in the tube, this time with 15 cc of each ether; shake vigorously 30 seconds after each addition; and allow to settle. Draw off the clear soln thru the small filter into the same flask as before and wash the tip of the spigot, the funnel, and the filter with a few cc of a mixture of the two ethers, in equal parts, free from suspended  $\text{H}_2\text{O}$ . To insure complete removal of the fat, a third extraction is necessary. This third extraction yields less than 1 mg of fat if the previous ether-fat solns have been drawn off closely. Add a glass bead and evaporate the ethers slowly on a steam bath; then dry the fat in a boiling water oven to constant weight. Weigh the flask with a similar flask as a counterpoise. Do not wipe the flask immediately before weighing.

Remove the fat completely with petroleum ether. Deduct the weight of the dried flask with residue and bead to obtain weight of fat. Finally, correct this weight by a blank determination on the reagents used.



*Babcock Method\*—Official*

## 20

## APPARATUS

(a) *Standard Babcock test milk bottle.*—8-g, 18-g, 6-inch milk-test bottle, total height 150–165 mm (5.9–6.5 inches). The bottom of the bottle shall be flat, and the axis of the neck shall be vertical when the bottle stands on a level surface. The charge of milk for the bottle shall be 18 g.

*Bulb.*—The capacity of the bulb to the junction with the neck shall be not less than 45 cc. The shape of the bulb shall be either cylindrical or conical. If cylindrical, the outside diameter shall be between 34 and 36 mm; if conical, the outside diameter of the base shall be between 31 and 33 mm, and the maximum diameter between 35 and 37 mm.

*Neck.*—The neck shall be cylindrical and of uniform diameter from at least 5 mm below the lowest graduation mark to at least 5 mm above the highest. The top of the neck shall be flared to a diameter of not less than 10 mm. The graduated portion of the neck shall have a length of not less than 63.5 mm. The total per cent graduation shall be 8. The graduations shall represent whole per cent, 0.5%, and 0.1%, respectively, from 0.0 to 8.0%. The tenths per cent graduations shall be not less than 3 mm in length; the 0.5% graduations shall be not less than 4 mm in length and shall project 1 mm to the left; and the whole per cent graduations shall extend at least half-way around the neck to the right and shall project at least 2 mm to the left of the tenths per cent graduations. Each whole per cent graduation shall be numbered, the number being placed to the left of the scale. The capacity of the neck for each whole per cent on the scale shall be 0.20 cc. The maximum error of the total graduation or any part thereof shall not exceed the volume of the smallest unit of the graduation.

Each bottle shall be so constructed as to withstand the stress to which it will be subjected in the centrifuge.

(a<sub>1</sub>) *Testing.*—The Hg and cork, alcohol and buret, and alcohol and brass plunger methods may be used for the rapid testing of the bottles, but the accuracy of any questionable bottle shall be determined by calibration with Hg (13.5471 g of clean, dry Hg at 20° to be equal to 5% on the scale of an 18-g bottle and 10% on the scale of a 9 g-bottle), the bottle being previously filled to zero with Hg.

(b) *Pipet.*—The standard milk pipet shall conform to the following specifications:

	mm
Total length, not more than.....	330
Outside diameter of suction tube.....	6–8
Length of suction tube.....	130
Outside diameter of delivery tube.....	4.5–5.5
Length of delivery tube.....	100–120
Distance of graduation mark above bulb.....	15–45
Nozzle, straight	
Graduation, to contain 17.6 cc of H <sub>2</sub> O at 20° when the bottom of the meniscus coincides with the mark on the suction tube.	
Delivery, 5–8 seconds.	
Maximum error in graduation, not to exceed 0.05 cc.	
The pipet is to be marked "Holds 17.6 cc."	

(b<sub>1</sub>) *Testing.*—The pipet shall be tested by measuring from a buret the volume of H<sub>2</sub>O (at 20°) which it holds up to the graduation mark.

(c) *Acid measure.*—The device used to measure  $H_2SO_4$ , whether a graduated cylinder or a pipet attached to a Swedish acid bottle, shall be graduated to deliver 17.5 cc.

(d) *Centrifuge or "tester."*—The standard centrifuge, however driven, shall be constructed thruout and so mounted as to be capable, when filled to capacity, of rotating at the necessary speed with a minimum of vibration and without liability of causing injury or accident. It shall be heated, electrically or otherwise, to a temp. of at least  $55^\circ$  during the process of centrifuging. It shall be provided with a speed indicator, permanently attached, if possible. The proper rate of rotation may be ascertained by reference to the table below. By "diameter of wheel" is meant the distance between the inside bottoms of opposite cups measured thru the center of rotation of the centrifuge wheel while the cups are horizontally extended.

Diameter of wheel, inches:	10	12	14	16	18	20	22	24
No. revolutions per minute:	1074	980	909	848	800	759	724	693

(e) *Dividers or calipers.*—For measuring the fat column.

(f) *Water bath for test bottles.*—Provided with a thermometer and a device for maintaining a temp. of  $55-60^\circ$ .

## 21

## DETERMINATION

Transfer 18 g of the prepared sample, 2, to the milk-test bottle by means of the pipet. Blow out the milk remaining in the pipet tip after free outflow has ceased. Add 17.5 cc of  $H_2SO_4$  (sp. gr. 1.82–1.83 at  $20^\circ$ ), preferably not all at one time, pouring it down the side of the neck of the bottle in such a way as to wash all traces of the milk into the bulb. The temp. of the acid shall be about  $15-20^\circ$ . Shake until all traces of curd have disappeared; then transfer the bottle to the centrifuge; counter-balance it; and, after the proper speed has been attained, whirl 5 min. Add soft  $H_2O$  at  $60^\circ$ , or above, until the bulb of the bottle is filled. Whirl 2 min. Add hot  $H_2O$  until the liquid column approaches the top graduation of the scale. Whirl 1 min. longer at a temp. of  $55-60^\circ$ . Transfer the bottle to the warm water bath maintained at a temp. of  $55-60^\circ$ , immerse it to the level of the top of the fat column, and leave it there until the column is in equilibrium and the lower fat surface has assumed a final form. Remove the bottle from the bath, wipe it, and with the aid of dividers or calipers measure the fat column, in terms of percentage by weight, from its lower surface to the highest point of the upper meniscus.

The fat column, at the time of measurement, should be translucent, of a golden yellow or amber color, and free from visible suspended particles. Reject all tests in which the fat column is milky or shows the presence of curd or of charred matter, or in which the reading is indistinct or uncertain.

## ADDED WATER

## 22

*Acetic Serum Method*—Official

(a) *Zeiss immersion refractometer reading.*—To 100 cc of the milk, measured at  $20^\circ$  into a beaker, add 2 cc of 25% acetic acid (sp. gr. 1.035). Cover the beaker with a watch-glass and place in a water bath at  $70^\circ$  for 20 min. Place the beaker in ice  $H_2O$  for 10 min. and separate the curd from the serum by rapid filtration thru a small filter. Transfer a portion of the clear serum to a refractometer beaker, place in the constant temp. bath, and take the refractometer reading when the temp. of the serum has been brought to exactly  $20^\circ$ , as determined by a thermometer graduated in tenths of a degree. A reading below 39 indicates added  $H_2O$ ; between 39 and 40,

the addition of  $H_2O$  is suspected. When the reading is 40 or below, determine the ash in the serum as directed under (b).

(b) *Ash*.—Transfer 25 cc of the serum to a weighed flat-bottomed Pt dish and evaporate to dryness on a water bath. Heat over a low flame (to avoid spattering) until the contents are thoroly charred, place the dish in an electric muffle, preferably with pyrometer attached, and ignite to a white ash at a temp. not greater than  $500^\circ$ . Cool and weigh. Express the result as grams per 100 cc. A result below 0.715 g per 100 cc indicates added  $H_2O$ . The acetic serum ash  $\times$  the factor 1.021 = the sour serum ash (dilution of the acetic serum being 2%).

## 23

*Sour Serum Method—Official*

(a) *Zeiss immersion refractometer reading*.<sup>8</sup>—Allow the milk to become completely sour, filter, and determine the immersion refractometer reading of the clear serum at  $20^\circ$ . A reading below 38.3 indicates added  $H_2O$ .

(b) *Ash*.<sup>2</sup>—Determine the ash in 25 cc of the serum obtained in (a) as directed under 22(b). A result below 0.730 g per 100 cc indicates added  $H_2O$ .

## 24

*Copper Serum Method<sup>10</sup>—Official*

To 1 volume of  $CuSO_4$  soln (72.5 g of  $CuSO_4 \cdot 5H_2O$  per liter, adjusted if necessary to read 36 at  $20^\circ$  on the scale of the Zeiss immersion refractometer, or to a specific gravity of 1.0443 at  $20/4^\circ$ ), add 4 volumes of milk. Shake well and filter. Determine the refractometer reading of the clear serum at  $20^\circ$ . A reading below 36 indicates added  $H_2O$ . When the refractometer reading is 36 or below, determine the ash of the sour serum as directed under 23(b) or of the acetic serum as directed under 22(b).

*Cryoscopic Method<sup>11</sup>—Official*

## 25

## APPARATUS

(a) *Cryoscope*.—A cylindrical-shaped Dewar flask of 1 liter capacity and 28 cm internal depth, surrounded by a metal casing, is tightly closed by means of a large cork of about 3 cm thickness. Thru the center of the cork is tightly fitted a medium thin-walled glass or metal tube, 250 mm in length by 33 mm outside diameter. At one side of the cork is inserted a narrow metal inlet tube, the lower end of which is formed into a perforated loop near the bottom of the flask. At the opposite side is a metal tube of T-shape construction and 6 mm internal diameter, intended to afford escape for vapors, and also for introducing volatile fluid into the apparatus. At the back portion of the cork is fitted a control thermometer, the bulb of which extends nearly to the bottom of the flask. The freezing test tube is of thin glass, about 240 mm in length by 29 mm outside diameter, and fits closely into the larger tube, which is sealed into the cork. In the rubber stopper of the freezing tube is fitted the standard thermometer. The length of the thermometer permits insertion of the bulb nearly to the bottom of the tube and at the same time allows complete exposure of the scale above the stopper. At the right side of the thermometer a stirring device made of non-corrodible low conductivity metal is fitted into the stopper thru a short section of thin-walled metal tubing. The lower end extends nearly to the bottom of the test tube and is provided with a horizontal loop encircling the thermometer. At the left of the thermometer is a freezing-starter attachment inserted thru an opening in the stopper formed by means of a short section of metal tubing. This device consists of a non-corrodible metal rod, at the lower end of which is a 10 mm length opening for the purpose of carrying a small fragment of

ice. At one side of the cryoscope is installed an air-drying arrangement which consists of a Folin absorption bulb inserted thru a tightly fitting stopper and extending nearly to the bottom of a large-sized test tube. A short section of glass tubing is inserted thru a second opening in the stopper and is connected to the vaporizing tube which enters the cryoscope. Sulfuric acid is poured into the drying tube to a level slightly above the small inner bulb. At the opposite side of the apparatus is arranged a drain tube for the purpose of conducting vapors away from the operator. By means of a pressure and suction pump dry air may be forced into the apparatus at a suitable rate and the mixed vapors conducted out thru the base of the drain tube into the sink. An adjustable lens is mounted in a convenient position in front of the thermometer for the purpose of magnifying the scale.

(b) *Standard thermometer*.—A solid-stem instrument having a total length of 58 cm, with a scale portion measuring about 30 cm. The total scale range is  $3^{\circ}$ , from  $+1^{\circ}$  to  $-2^{\circ}$ , and each degree division is subdivided into tenths and hundredths. The length of a degree division approximates 1 dm, thus making the smallest subdivisions of such magnitudes as to enable easy observation and readings estimated to  $0.001^{\circ}$ . Standardize the thermometer as directed under 26. Check at frequent intervals, once a week or as often as may be necessary, to keep an accurate record of any changes that may occur.

(c) *Control thermometer*.—A solid-stem instrument approximately 58 cm in length and having a scale range of  $+20^{\circ}$  to  $-30^{\circ}$ . Test in a bath of melting crushed ice for the purpose of determining whether the 0-mark on the scale is correct. The scale graduations should be accurate to within  $0.10^{\circ}$ .

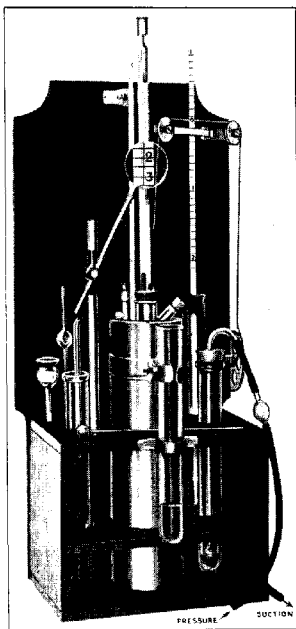


FIG. 24.—HORTVET CRYOSCOPE

## 26

### STANDARDIZATION OF THE THERMOMETER

Make 3 freezing-point determinations by the procedure given under 27 on each of the following:

(a) *Recently boiled distilled  $H_2O$* .

(b) *Sucrose soln*.—Dissolve 7 g of pure sucrose in  $H_2O$  and make the soln to a volume of 100 cc at  $20^{\circ}$ .

(c) *Sucrose soln*.—Dissolve 10 g of pure sucrose in  $H_2O$  and make the soln to a volume of 100 cc at  $20^{\circ}$ .

(A sample of pure sucrose may be obtained by application to the Director of the Bureau of Standards, Department of Commerce, Washington, D. C.)

Tabulate the results in the following form:

FREEZING- POINT OBSERVATIONS	PURE WATER	7 GRAMS SUCROSE SOLUTION		10 GRAMS SUCROSE SOLUTION	
		Observed freezing point (-S)	Freezing-point depression S - W (algebraic)	Observed freezing point (-S)	Freezing-point depression S - W (algebraic)
1st					
2nd					
3rd					
Averages	$\pm W$	XXXXXXX		XXXXXXX	

Express the results as degrees freezing-point depression below the average of the observed freezing points obtained on the sample of pure  $H_2O$  ( $\pm W$ ), which may be above (+) or below (-) the 0-mark on the scale. Obtain each freezing-point depression of the sucrose solns by the algebraic subtraction of the average of the freezing-point readings of pure  $H_2O$  ( $\pm W$ ) from each observed freezing point.

Omit adventitious results, i.e., results which are in marked disagreement with other results obtained by carefully following instructions.

Apply the average of the freezing-point depressions obtained on the standard sucrose solns for the purpose of correcting the thermometer readings obtained on samples of milk in the manner illustrated in the tables accompanying Fig. 25.

## 27

## DETERMINATION

(Make freezing-point determinations only on samples of milk that show an acidity of not more than 0.18% when determined as directed under 4.)

Insert the funnel-tube into the vertical portion of the T-tube at one side of the apparatus and pour in 400 cc of ether previously cooled to  $10^\circ$  or lower. Close the vertical tube by means of a small cork and connect the pressure pump to the inlet tube of the air-drying attachment. Adjust the pump so as to pass air thru the apparatus at a moderate rate, as may be judged by the agitation of the  $H_2SO_4$  in the drying tube. Continuous vaporization of the ether will cause a lowering of the temp. in the flask, from ordinary room temp. to  $0^\circ$  in from 5 to 10 min. Continue the temp. lowering until the control thermometer registers near  $-3^\circ$ . At this stage, by lowering the gage tube into the ether bath, then closing the top by means of the forefinger and raising to a suitable height, an estimate can be made as to the quantity of ether necessary to pour in for the purpose of restoring the 400 cc volume. When the volume of ether has been adjusted to 400 cc an additional 10-15 cc is sufficient on an average for each succeeding determination. Pour into the freezing test tube sufficient  $H_2O$  (30-35 cc), boiled and cooled to  $10^\circ$  or lower, to submerge the thermometer bulb. Insert the thermometer together with the stirrer and lower the test tube into the larger tube. A small quantity of alcohol, sufficient to fill the lower space between the 2 test tubes, will serve to complete the conduction medium between the freezing bath and the liquid to be tested. Keep the stirrer in steady up-and-down motion at a rate of approximately one stroke each 1 or 2 seconds, or even at a slower rate, providing the cooling proceeds satisfactorily. Maintain a passage of air thru the apparatus until the temp. of the cooling-bath reaches  $-2.5^\circ$ , at which time the top of the Hg thread in the thermometer usually recedes to a position near the freezing point of  $H_2O$ . Maintain the temp. of the cooling-bath at  $-2.5^\circ$  and continue the manipulation of the stirrer until a super-cooling of sample

of 1.0 to 1.2° is observed. As a rule, at this time the liquid will begin to freeze, as may be noted by the rapid rise of the Hg. Manipulate the stirrer slowly and carefully 3 or 4 times as the Hg column approaches its highest point. By means of a suitable light-weight cork mallet tap the upper end of the thermometer cautiously a number of times until the top of the Hg column remains stationary for at least 1 min. Observe the exact reading on the thermometer scale, taking necessary precautions to avoid parallax, and estimate to 0.001°. When the observation has been

Two Bureau of Standards tested thermometers gave intervals of 0.199° and 0.200°, respectively, between the freezing-point depression readings of the two sucrose solns. One thermometer gave freezing-point depressions -0.422° and -0.621°, respectively, for the two sucrose solns, while the other gave -0.422° and -0.622°, respectively.

Laboratory Thermometer No. 2.

WATER	7 GRAMS SUCROSE TO 100 cc	10 GRAMS SUCROSE TO 100 cc
+0.056°	-0.425°	-0.621°

Interval = 0.196  
0.196 equiv. 0.199  
Correction =  $\times 1.015$

Laboratory Thermometer No. 24.

WATER	7 GRAMS SUCROSE TO 100 cc	10 GRAMS SUCROSE TO 100 cc
0.000°	-0.420°	-0.625°

Interval = 0.205  
0.205 equiv. 0.199  
Correction =  $\times 0.971$

*Example:*

Laboratory Thermometer No. 24.  
F. pt. Depression Sample Milk = 0.548  
(0.548 - .420) 0.971 = 0.124  
Corrected Depression = 0.422 + 0.124  
= 0.546°

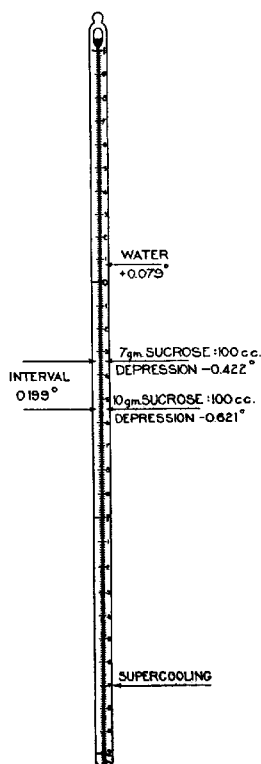


FIG. 25.—U. S. BUREAU OF STANDARDS TESTED THERMOMETER

satisfactorily completed, make a duplicate determination; then remove the thermometer and stirrer and empty the  $H_2O$  from the freezing tube.

Rinse the tube with about 25 cc of the sample of milk, cooled to  $10^\circ$  or lower; measure into the tube 30–35 cc of milk, or enough to submerge the thermometer bulb; and insert the tube into the apparatus. Maintain the temp. of the cooling-bath at  $2.5^\circ$  below the probable freezing point of the sample. Make the determination on the milk, following the same procedure as that used in determining the freezing point of  $H_2O$ . As a rule, however, it is necessary to start the freezing action in the milk by inserting the freezing starter (which has been kept in contact with ice for several minutes, and in the open end of which has been wedged a fragment of ice) at the time when the Hg column has receded to  $1.0$ – $1.2^\circ$  below the probable freezing point. A rapid rise of the Hg results almost immediately. Remove the starter and manipulate the stirrer slowly and carefully 2 or 3 times while the Hg approaches its highest point. Complete the adjustment of the Hg column in the same manner as in the preceding determination; then, avoiding parallax, observe the exact reading on the thermometer scale and estimate to  $0.001^\circ$ . The algebraic difference between the average of readings obtained on the  $H_2O$  and the reading obtained on the sample of milk represents the *freezing-point depression* of the milk. Apply necessary correction to the result in the manner shown in the illustrative tables accompanying Fig. 25.

Ascertain the percentage of added  $H_2O$  corresponding to the determined freezing-point depression from Table 22, XLII. The percentage of added  $H_2O$  ( $W$ ) may also be calculated as follows:

$$W = \frac{100(T - T^1)}{T}, \text{ in which}$$

$T$  = the average freezing point of normal milk ( $-0.550^\circ$ ), and

$T^1$  = the observed freezing point on a given sample.

A tolerance of 3% may be allowed on results for added  $H_2O$  determined on the basis of an average freezing-point depression of  $-0.550^\circ$ . Owing to the narrow variations found in market milks of genuine character it is not necessary to deduct the tolerance figure from results showing added  $H_2O$  in excess of 3%.

#### GELATIN<sup>12</sup>

28

##### *Qualitative Test—Official*

To 10 cc of the milk add an equal volume of acid  $Hg(NO_3)_2$  soln (Hg dissolved in twice its weight of  $HNO_3$  and this soln diluted to 25 times its volume with  $H_2O$ ), shake the mixture, add 20 cc of  $H_2O$ , shake again, allow to stand 5 min., and filter. If much gelatin is present, the filtrate will be opalescent and cannot be obtained quite clear. To a portion of the filtrate contained in a test tube add an equal volume of saturated aqueous picric acid soln. A yellow precipitate will be produced in the presence of any considerable quantity of gelatin, while smaller quantities will be indicated by a cloudiness.

NOTE: In applying this test to sour, fermented, cultured, or very old samples of milk, cream, or buttermilk; to sterilized cream or evaporated milk; or to cottage cheese, use care to recognize precipitates produced by picric acid when added to the  $Hg(NO_3)_2$  filtrates from these materials in the absence of gelatin. (Such samples, with or without rennet and entirely free from gelatin, give, on standing, distinct precipitates when treated as above outlined. In every case, however, these precipitates differ in character from that which picric acid produces with gelatin. The

gelatin-picric acid precipitate is finely divided, more apt to remain in suspension, settles only slowly, and adheres tenaciously to the sides and bottom of the container from which it is rinsed with difficulty. Precipitates produced by picric acid in the absence of gelatin are flocculent, separate readily, leaving the serum practically clear, do not adhere to the walls of the container, and are easily removed by rinsing with  $H_2O$ . When gelatin is present in the sample, the gelatin-picric acid precipitate will remain in suspension long after the flocculent precipitate has settled, but on standing overnight the characteristic sticky deposit will be found adhering tenaciously to the bottom and sides of the test vessel. If gelatin is present in relatively high concentration (1%), the gelatin-picric acid precipitate will be voluminous and will settle rather quickly.)

When examining cottage cheese, mix thoroly 5 g of the sample with 10 cc of  $H_2O$  at 50-60° and add 5 cc of  $Hg(NO_3)_2$  soln. Shake, let stand 5 min., and filter thru a medium fast, retentive paper. To the filtrate add 5 cc more of the  $Hg(NO_3)_2$  soln and test as before, using the filtrate so obtained for the test.

## 29

## PRESERVATIVES--OFFICIAL

Proceed as directed under XXXII. To test for salicylic acid or benzoic acid acidify 100 cc of the milk with 5 cc of  $HCl$  (1+3), shake until curdled, filter, and treat the clear filtrate as directed under XXXII, 2, 3, or 6.

To test for  $HCHO$  proceed as directed under XXXII, 19-25, inclusive, applying the test directly to the milk.

## 30

## COLORING MATTERS--OFFICIAL

Warm about 150 cc of milk in a casserole over a flame and add about 5 cc of acetic acid (1+3), then slowly continue the heating nearly to the boiling point while stirring. Gather the curd, when possible, into one mass with a stirring rod and pour off the whey. If the curd breaks up into small flecks, separate from the whey by straining thru a sieve or colander. Press the curd free from adhering liquid, transfer to a small flask, macerate with about 50 cc of ether, keeping the flask tightly corked and shaking at intervals, and allow to stand for several hours, preferably overnight. Decant the ether extract into an evaporating dish, remove the ether by evaporation, and test the fatty residue for annatto as directed under XXI, 16(b).

The curd of an uncolored milk is perfectly white after complete extraction with ether, as is also that of a milk colored with annatto. If the extracted fat-free curd is distinctly orange or yellowish in color, a coal tar dye is indicated. In many cases if a lump of a fat-free curd in a test tube is treated with a little  $HCl$  the color changes to pink, indicating the presence of a dye similar to aniline yellow or butter yellow or perhaps one of the acid azo yellows or oranges. In such cases, separate and identify the coloring matter present in the curd as directed under XXI, 11. If aniline yellow, butter yellow, or any other oil-soluble dye is present, the greater part will be found in the ether extract containing the fat. In such cases proceed as directed under XXI, 3.

In some cases the presence of coal tar dyes can be detected by treating about 100 cc of the milk directly with an equal volume of  $HCl$  in a porcelain casserole, giving the dish a slight rotary motion. In the presence of some dyes the separated curd acquires a pink coloration.

SEDIMENT TEST<sup>13</sup>

(Taken from Standard Methods of Milk Analysis, American Public Health Association. This method has been edited to conform in part to the style of this publication.)



## 31

## SAMPLING

Pint samples only are regarded as standard. If quart or any other size of sample is used, report the size used. Take these samples from well-stirred cans or vats of milk or from pint or well-shaken quart bottles of milk. Do not take samples from the unstirred bottom milk of 40-quart or other cans. Measure the amount of milk used with reasonable accuracy.

## 32

## PREPARATION OF SEDIMENT DISKS

Strain the pint sample of milk thru a suitable sediment tester fitted with a firm cotton disk (the type furnished by the Lorenz Model Co., Madison, Wis., is suitable) placed over an opening 1 in. in diameter. (It will hasten the process of filtering if provision is made for warming the milk, or the milk may be forced thru the disk by air pressure.)

## 33

## PREPARATION OF STANDARD SEDIMENT DISKS

Prepare a suspension of weathered, dried, finely ground cow dung, using a 50% cane sugar soln as the medium.

Dry the cow dung in an oven and grind thru a laboratory feed mill several times (practically all the ground material should be fine enough to pass a 60-mesh screen, and the greater portion should be finer than 100-mesh). Accurately weigh 0.1 g and transfer to a 1000 cc measuring flask, using the 50% sugar soln to wash all the fine particles down into the flask. Make the volume up to the 1000 cc mark with more of the sugar soln after most of the fine particles have been wetted by shaking the half-filled flask thoroly several times. After the volume is made up to the mark, shake the contents of the flask vigorously every 5 min. for sufficient time to saturate the particles thoroly ( $\frac{1}{2}$ -1 hr.). When the particles have been thoroly wetted it will be noted that the sugar soln will hold them evenly in suspension, and the mixture is ready to use in making the standard disks.

On the basis of 0.1 g per 1000 cc, 10 cc of the sugar soln contains 1 mg of sediment. Make test disks with one of the usual sediment testers, using varying volumes of the sediment suspension. Place several ounces of filtered skimmed milk in the sediment tester and add varying volumes of the sediment suspension. After forcing the milk thru the disks, run thru a small quantity of filtered skimmed milk to obtain a more even distribution of the sediment on the disk.

Remove the disk from the tester, mount permanently on a stiff paper, allow to dry, and then make permanent by spraying with a strong disinfectant such as corrosive sublimate. A good apparatus for this purpose is an ordinary throat atomizer, provided caution is observed not to use corrosive sublimate in contact with metal. Below each mounted standard disk on the paper note the quantity of dried material that the dirt or filth on the disk represents.

**NOTE:** An excellent set of standard disks has been prepared and photographed by the Connecticut State Department of Health Laboratory. Thru the courtesy of the Connecticut laboratory, photographic copies of these standard disks may be secured thru the office of the American Public Health Association, 50 West 50th St., New York City.

The standards given are based on pint samples of milk to which weighed amounts of sediment have been added, and cover the entire range from "clean" to "very dirty" milk. Numerical ratings are given for the convenience of those who wish to use these standard disks as the basis of percentage or other numerical scores. No attempt should be made to grade as sediment any hair, piece of hay or straw, or any large particle of dirt. These should be reported separately.

## CREAM

## 34 COLLECTION OF SAMPLE—OFFICIAL

Proceed as directed under 1. Analyze the sample as soon as practicable, preferably not later than 3 days after taking.

## 35 PREPARATION OF SAMPLE—OFFICIAL

Immediately before withdrawing portions for the determinations, mix the sample by shaking, pouring, or stirring until it pours readily and a uniform emulsion has been secured. If the sample is very thick, warm it to 30–35°, and then mix. In case lumps of butter have separated, heat the sample to 38° or, if necessary, to 50°, by placing in a warm water bath. Thoroughly mix the portions for analysis and weigh immediately. (In commercial testing for fat by the Babcock method, it may be advisable to warm all samples to 38–50° in a water bath previous to mixing.) Avoid overheating the sample, thereby causing the cream to “oil off.” (This precaution is especially necessary in the case of a thin cream.)

## 36 TOTAL SOLIDS—OFFICIAL

Proceed as directed under 8, using 2–3 g of the sample.

37 ADDED WATER IN CREAM<sup>1</sup>—OFFICIAL

Proceed as directed under 25, but use the following formula to calculate the percentage of added H<sub>2</sub>O:

$$W = \frac{\% \text{ Serum in Cream } (T - T')}{T}, \text{ in which}$$

$W$  = the percentage of added H<sub>2</sub>O;

$T$  = the freezing point of undiluted cream (−0.550°);

$T'$  = the observed freezing point of the given sample; and

$\% \text{ Serum} = 100\% - (\% \text{ fat} + \% \text{ protein}).$

If protein is not determined it may be assumed to be 38% of the solids-not-fat.

## 38 ASH—OFFICIAL

Proceed as directed under 9.

## 39 TOTAL NITROGEN—OFFICIAL

Proceed as directed under 10.

## LACTOSE

## 40 Gravimetric Method—Official

Proceed as directed under 18.

## FAT

41 *Rosse-Gottlieb Method—Official*

Transfer 5 g of the sample to a Röhrig tube or a similar apparatus, dilute with H<sub>2</sub>O to about 10.5 cc, and proceed as directed under 19.

*Babcock Method<sup>2</sup>—Official*

## 42 APPARATUS

(a) *Test bottles.*—The standard Babcock test bottles for cream shall be as follows:

(1) 50%, 9-g, short-necked, 6-inch cream-test bottle.—Total height 150–165 mm (5.9–6.5 inches). The bottom of the bottle shall be flat, and the axis of the neck shall be vertical when the bottle stands on a level surface. The charge of cream for the bottle shall be 9 g.

*Bulb*.—The capacity of the bulb to the junction with the neck shall be not less than 45 cc. The shape of the bulb shall be either cylindrical or conical. If cylindrical, the outside diameter shall be between 34 and 36 mm; if conical, the outside diameter of the base shall be between 31 and 33 mm, and the maximum diameter between 35 and 37 mm.

*Neck*.—The neck shall be cylindrical and of uniform diameter from at least 5 mm below the lowest graduation mark to at least 5 mm above the highest. The top of the neck shall be flared to a diameter of not less than 15 mm. The graduated portion of the neck shall have a length of not less than 63.5 mm. The total per cent graduation shall be 50. The graduations shall represent 5%, 1%, and  $\frac{1}{2}\%$ , respectively, from 0.0 to 50%. The 5% graduations shall extend at least half-way around the neck to the right; the  $\frac{1}{2}\%$  graduations shall be not less than 3 mm in length; and the 1% graduations shall be intermediate in length between the 5% and  $\frac{1}{2}\%$  graduations and shall project 2 mm to the left of the  $\frac{1}{2}\%$  graduations. Each 5% graduation shall be numbered (thus: 0, 5, 10, . . . 45, 50), the number being placed to the left of the scale. The capacity of the neck for each whole per cent on the scale shall be 0.1 cc. The maximum error in the total graduation or any part thereof shall not exceed the volume of the smallest unit of the graduation.

(2) *50%, 9-g, long-necked, 9-inch cream-test bottle*.—The same specifications shall apply to this bottle as to the 50%, 9-g, 6-inch cream-test bottle, except that the total height of this bottle shall be 210–229 mm (8.25–9.0 inches) and the graduated portion of the neck shall have a length of not less than 120 mm.

(3) *50%, 18-g, long-necked, 9-inch cream-test bottle*.—The same specifications shall apply to this bottle as to the 50%, 9-g, 9-inch cream-test bottle, except that the charge of cream for this bottle shall be 18 g.

Each bottle shall bear on the top of the neck above the graduations, in plain legible characters, a mark denoting the weight of the charge to be used, *viz.*, “9 g” or “18 g,” as the case may be.

Each bottle shall be so constructed as to withstand the stress to which it will be subjected in the centrifuge.

(4) *Testing*.—Proceed as directed under 20(a).

(b) *Water bath for cream samples*.—Provided with a thermometer and a device for maintaining a temp. of 38–50°.

(c) *Cream weighing scales*.—With a sensibility reciprocal of 30 mg, *i.e.*, the addition of 30 mg to either pan of the scale, when loaded to capacity, shall cause a deflection of at least 1 subdivision of the graduation. The scales shall be set level upon a table support and be protected from drafts.

(d) *Weights*.—9 g and 18 g, respectively, and plainly marked “9 g” or “18 g,” as the case may be. They shall be made of material capable of resisting corrosion or other injury, shall preferably be of a low squat shape, with rounded edges, and shall be verified at frequent intervals by comparison with standardized weights.

(e) *Acid measure*.—Described under 20(c).

(f) *Centrifuge or “tester”*.—Described under 20(d).

(g) *Dividers or calipers*.—Described under 20(e).

(h) *Water bath for test bottles*.—Described under 20(f).

## 43 •

## DETERMINATION

Weigh 9 g of the prepared sample, 35, directly into a 9-g cream-test bottle, or 18 g into an 18-g bottle, and proceed by one of the following methods:

*Method 1*.—After the cream has been weighed into the test bottle, add 8–12 cc of  $\text{H}_2\text{SO}_4$  (sp. gr. 1.82–1.83 at 20°) in the case of the 9-g bottle, or 14–17 cc of the

acid in the case of the 18-g bottle, or add acid until the mixture of cream and acid, after shaking, has assumed a chocolate-brown color. Shake until all lumps have completely disappeared; then add 5-10 cc of soft  $H_2O$  at  $60^\circ$  or above. Transfer the bottle to the centrifuge, counterbalance it, and after the proper speed has been attained whirl 5 min. Add soft hot  $H_2O$  until the liquid column approaches the top graduation of the scale; then whirl 1 min. longer at a temp. of  $55-60^\circ$ . Adjust the temp. as directed under 21, and with the aid of dividers or calipers measure the fat column, in terms of percentage by weight, from its lower surface to the bottom of the upper meniscus.

*Method 2.—For a 9-g bottle only.*—After the cream has been weighed into the test bottle, add 9 cc of soft  $H_2O$  and thoroly mix; add 17.5 cc of the  $H_2SO_4$  and shake until all lumps have completely disappeared. Transfer the bottle to the centrifuge, counterbalance it, and after the proper speed has been attained whirl 5 min. Fill the bottle to the neck with hot  $H_2O$  and whirl 2 min. Add hot  $H_2O$  until the liquid column approaches the top graduation of the scale; then whirl 1 min. longer at a temp. of  $55-60^\circ$ . Adjust the temp. and measure the fat column as directed under *Method 1*.

Whichever method is followed, the fat column, at the time of reading, should be translucent, of a golden yellow to amber color, and free from visible suspended particles. All tests in which the fat column is milky or shows the presence of curd or of charred matter, or in which the reading is indistinct or uncertain, should be rejected.

If glymol or pure white mineral oil (sp. gr. not to exceed 0.85 at  $20^\circ$ ) is used, introduce a few drops only into the bottle just before the reading is made (it must not be dropped in, but must be allowed to flow down the side of the neck). For the purpose of measurement, the surface separating the glymol and the fat is regarded as representing the upper limit of the column. Oil-soluble artificial color may be added to white mineral oil.

#### 44 GELATIN—OFFICIAL

Proceed as directed under 28.

#### 45 PRESERVATIVES—OFFICIAL

Proceed as directed under 29 and under XXXII.

#### 46 COLORING MATTERS—OFFICIAL

Proceed as directed under 30 and under XXI.

### EVAPORATED MILK (UNSWEETENED)

#### 47 PREPARATION OF SAMPLE—OFFICIAL

(a) Transfer the entire contents of a can to a large dish, stir thoroly, and pass thru a fine sieve or strainer until a homogeneous mass is secured. If a slight separation of fat is evident, warm a portion of the sample containing the separated fat to  $30-35^\circ$  and agitate until a uniform emulsion is obtained; then combine with the unheated portion and mix thoroly. (If an appreciable quantity of fat has separated, rendering impossible the formation of a satisfactory emulsion, an accurate analysis cannot be made.)

(b) Dilute 40 g of the prepared homogeneous mass (a) with 60 g of  $H_2O$  and mix thoroly.

#### 48 TOTAL SOLIDS—OFFICIAL

Weigh 4-5 g of the diluted sample, 47(b), into a weighed flat-bottomed Pt dish, not less than 5 cm in diameter, and proceed as directed under 8. Correct the result for the dilution.

**49 ASH—OFFICIAL**

Ignite the residue from the total solids determination, 48, at a low red heat until the ash is free from C. Correct the result for the dilution.

**50 FAT—OFFICIAL**

Weigh 4–5 g of the undiluted sample, 47(a), into a Röhrig tube or a similar apparatus; dilute with  $H_2O$  to about 10.5 cc; and proceed as directed under 19.

**51 TOTAL NITROGEN—OFFICIAL**

Weigh 5 g of the undiluted sample, 47(a), transfer to a Kjeldahl flask, and proceed as directed under II, 21, 23, or 25. The percentage of  $N \times 6.38$  = percentage of N compounds.

**52 CASEIN—OFFICIAL**

Weigh 10 g of the diluted sample, 47(b), into a beaker, and proceed as directed under 11 or 12. Correct the result for the dilution.

**53 ALBUMIN—OFFICIAL**

Proceed as directed under 14, using the filtrate from 52. Correct the result for the dilution.

**54 LACTOSE—OFFICIAL**

Proceed as directed under 16 or 18, using the diluted sample, 47(b), and correct the result for the dilution.

**55 GELATIN—OFFICIAL**

Proceed as directed under 28.

**56 PRESERVATIVES—OFFICIAL**

Proceed as directed under 29 and under XXXII.

**57 COLORING MATTERS—OFFICIAL**

Proceed as directed under 30 and under XXI.

**SWEETENED CONDENSED MILK****58 PREPARATION OF SAMPLE—OFFICIAL**

(a) If the can is cold, place it in  $H_2O$  at 30–35° until warm. Open, scrape out all milk adhering to the interior of the can, and after transferring to a dish sufficiently large to permit stirring thoroly mix until the whole mass is homogeneous.

(b) Weigh 100 g of the thoroly mixed sample into a 500 cc volumetric flask, dilute to the mark with  $H_2O$ , and mix thoroly. If the sample will not emulsify uniformly, weigh out a separate portion of (a) for each determination.

**59 TOTAL SOLIDS—OFFICIAL**

Use 10 cc of the prepared soln, 58(b), and proceed as directed under 8, drying on either sand or asbestos fiber. Correct the result for the dilution.

**60 ASH—OFFICIAL**

Evaporate 10 cc of the prepared soln, 58(b), to dryness on a water bath and ignite the residue as directed under XXVII, 8. Correct the result for the dilution.

61

## PROTEIN—OFFICIAL

Determine the N as directed under II, 21, 23, or 25, using 10 cc of the prepared soln, 58(b), and multiply by 6.38 to obtain the equivalent of protein. Correct the result for the dilution.

62

## LACTOSE—OFFICIAL

Dilute 100 cc of the prepared soln, 58(b), in a 250 cc volumetric flask to about 200 cc; add 6 cc of Fehling's  $\text{CuSO}_4$  soln, XXXIV, 31(a), make up to the mark, and mix thoroly. Filter thru a dry filter and determine lactose as directed under XXXIV, 57. Correct the result for the dilution.

63

## FAT—OFFICIAL

Weigh accurately 4–5 g of the prepared sample, 53(a), into a Röhrig tube or a similar apparatus; dilute with  $\text{H}_2\text{O}$  to about 10.5 cc, and proceed as directed under 19.

SUCROSE<sup>1a</sup>—OFFICIAL

64

## REAGENT

To 220 g of yellow  $\text{HgO}$ , add 300–400 cc of  $\text{H}_2\text{O}$  and sufficient  $\text{HNO}_3$  to form a clear soln (about 140 cc), being careful to use the least possible excess of acid. Dilute to 800–900 cc and add 10%  $\text{NaOH}$  soln slowly and with constant shaking until a slight permanent precipitate is obtained. Dilute to 1 liter and filter. As the soln tends to become acid with age owing to the deposition of basic mercuric salts, dilute alkali should be added occasionally until a slight permanent precipitate is formed, and the soln filtered.

65

## DETERMINATION

Introduce 50 cc of the prepared soln, 58(b), into a 100 cc volumetric flask; add 25 cc of  $\text{H}_2\text{O}$ , mix, add 5 cc of the  $\text{Hg}(\text{NO}_3)_2$ , and shake thoroly. Without delay and while shaking constantly, add sufficient 0.5  $N$   $\text{NaOH}$  soln to render the mixture neutral to litmus paper, being careful to avoid an alkaline reaction (usually 12–13 cc will be required). Dilute to 100 cc with  $\text{H}_2\text{O}$ , mix thoroly, and filter thru a dry paper. Polarize the filtrate in a 200 mm tube, then invert at room temp. as directed under XXXIV, 23(c), and polarize the inverted soln. Correct both readings for the volume occupied by the protein, 61, and the fat, 63, 1 g of protein occupying a space of 0.8 cc and 1 g of fat, 1.075 cc. Calculate the percentage of sucrose by the following formula, using the corrected direct and invert readings obtained above:

$$S = \frac{100(a-b)}{142.35 - \frac{t}{2}} \times \frac{26}{W}, \text{ in which}$$

$S$  = percentage of sucrose in the sample

$a$  = corrected direct polarization,

$b$  = corrected invert polarization,

$t$  = temp. of soln polarized; and

$W$  = weight of sample taken (10 g).

## DRIED MILK AND MALTED MILK

66

SAMPLING DRIED MILK<sup>1a</sup>—TENTATIVE

Use care to minimize any moisture absorption from the air during sampling and avoid sampling on a rainy day, or when the humidity is high.

On the surface of the milk at the top of the barrel locate a point on each end of a diameter and on a radius perpendicular to the diameter one to two inches in from the edge of the barrel. Midway on each side of the triangle between these points locate a point. At the six points so located, using a tubular trier sufficiently long to extend the full length of the barrel, draw a core parallel to the vertical axis of the barrel. Transfer the cores to a clean, dry, air-tight container and seal immediately.

Before opening the sample for analysis, make homogeneous either by shaking or by alternately rolling and inverting the container. Also avoid excessive temp. and humidity when opening the sample container.

67

**PREPARATION OF SAMPLE—TENTATIVE**

Sift the sample thru a 20-mesh sieve onto a large sheet of paper, rubbing the material thru the sieve and tapping vigorously if necessary. Grind the residue in a mortar, pass thru the sieve, and mix into the sifted material. Discard particles of wood and other material that cannot be ground. Sift the sample 2 more times, mixing thoroly each time. To avoid absorption of moisture, operate as rapidly as possible, and preserve the sample in an air-tight container.

**MOISTURE<sup>17</sup>—TENTATIVE**

68

**DETERMINATION**

Weigh 1–1.5 g of the sample into a previously weighed metal dish (diameter about 55 mm, height about 15 mm, provided with a slip-in inverted cover fitting tightly on the inside), cover tightly, and reweigh. Place the loosely covered dish in direct contact with the metal shelf of the vacuum oven and dry to constant weight (approximately 5 hours) under a pressure not to exceed 100 mm (4 inches) of Hg, at the temp. of boiling H<sub>2</sub>O. During the drying admit into the oven a slow current of air (about 2 bubbles per second), dried by passing thru H<sub>2</sub>SO<sub>4</sub>. Discontinue the action of the vacuum pump and carefully admit dried air into the oven. Press the cover tightly into the dish, remove the dish from the oven, cool, and weigh. Calculate the percentage loss in weight as moisture.

69

**PROTEIN—TENTATIVE**

Weigh 1 g of the sample into a Kjeldahl digestion flask and determine N as directed under II, 25. The % of N  $\times 6.38 =$  % of N compounds.

70

**ASH—TENTATIVE**

Ignite 1 g of the sample at a low red heat until free from C. Cool in a desiccator and weigh.

71

**FAT IN MALTED MILK<sup>18</sup>—TENTATIVE**

Weigh accurately about 1 g of the well-mixed sample into a small, lipped beaker. Add 1 cc of H<sub>2</sub>O and mix well with a glass rod to form a thick liquid free from lumps. Add 10 cc more of H<sub>2</sub>O, warm on the steam bath, and transfer to a Röhrig tube or similar apparatus. Cool, add 10 cc of 95% (by volume) alcohol, and mix. Add 25 cc of ethyl ether and proceed with the extraction as in the official Roesse-Gottlieb method for milk, 19. To obtain the weight of pure fat, proceed as directed under 19.

**FAT IN DRIED MILK<sup>19</sup>—OFFICIAL**

72

**PREPARATION OF SOLUTION**

Proceed as directed in one of the following methods:

(a) Weigh out about 1 g of well-mixed sample into a small, lipped beaker. Add 1 cc of H<sub>2</sub>O and mix well with a glass rod to form a thick liquid, free from lumps.

Add 9 cc more of  $H_2O$  and 1 cc of  $NH_4OH$  and warm on the steam bath. Transfer to a Röhrig tube or similar apparatus. Cool, and proceed as directed in 73, rinsing the beaker successively with the alcohol and ethers used in the first extraction.

(b) Weigh out about 1 g of well-mixed sample into a Röhrig tube or similar apparatus. Add 10 cc of  $H_2O$  and shake until homogeneous, warming if necessary. Add 1 cc of  $NH_4OH$  and heat in a water bath at 60–70° for 15 min., shaking occasionally. Cool, and proceed as directed in 73.

73

## DETERMINATION

Add 10 cc of 95% (by volume) alcohol, and mix. Extract with ethyl ether and petroleum ether as directed under 19. For the second extraction add 4 cc of 95% alcohol, and again extract as directed under 19. With whole milk and cream powders make a third extraction, using 15 cc of each ether after adding, if necessary, sufficient  $H_2O$  to raise the aqueous layer in the tube to its original volume. To obtain the weight of the pure fat, proceed as directed in 19.

74 MICROSCOPICAL IDENTIFICATION OF MALTED MILK AND ITS FLAVORED PRODUCTS<sup>20</sup>—TENTATIVE

Mount a small quantity of the material in a drop of mineral oil on a slide, apply the cover-glass, and examine the preparation at a magnification of approximately 200, using a microscope lamp with daylight glass as a source of light. Control the light intensity by the iris diaphragm because a too brilliantly lighted field hinders the recognition of details. (See pp. 284–287.)

## BUTTER

(These methods are also applicable to renovated or process butter and margarine.)

75

SAMPLING<sup>21</sup>—TENTATIVE

(a) *Tub or Cube Butter*.—Insert a regular trough butter trier practically its full length from a point near the top edge (or corner in case of a cube) thru the center to a point at the bottom diagonally opposite the point of entry. Give the trier one complete turn and withdraw a full core. Hold the point of the trier over the mouth of the sample container and immediately transfer the core of butter in approximately 3 in. sections, working it from the trier by the aid of a spatula fitted to the groove. Leave a plug about 1 in. long to place in the hole from which the core was removed. Add two other trierfuls taken similarly at points equidistant with the first (two other corners in case of a cube) to the jar to constitute a subdivision from the tub or cube sampled. Do not include moisture adhering to the outside of the trier. Clean and dry the trier before each drawing. Use an unwarmed trier for butter stored above the freezing point. For harder butter use a trier warmed to a temp. that may be just borne by the hand. Soften butter frozen so hard as to resist the trier by storage in a tempering room for 24 hours.

Sample lots as follows:

(1) *Tubs (or cubes) marked with churn numbers*.—Sample one tub of each churn of 1–9 tubs, two of each churn of 10–14 tubs, and three of each churn of over 14 tubs. In no case sample less than two tubs in a lot.

(2) *Tubs not marked with churn numbers*.—Sample a number of tubs equivalent to the square root of the number in the lot, with a minimum of 3 and a maximum of 25. If the square root is not a whole number, sample one extra tub.

(b) *Print butter*.—Withdraw one print from each of a number of cases equivalent to the square root of the number of cases in the lot, with a minimum of 5 and a



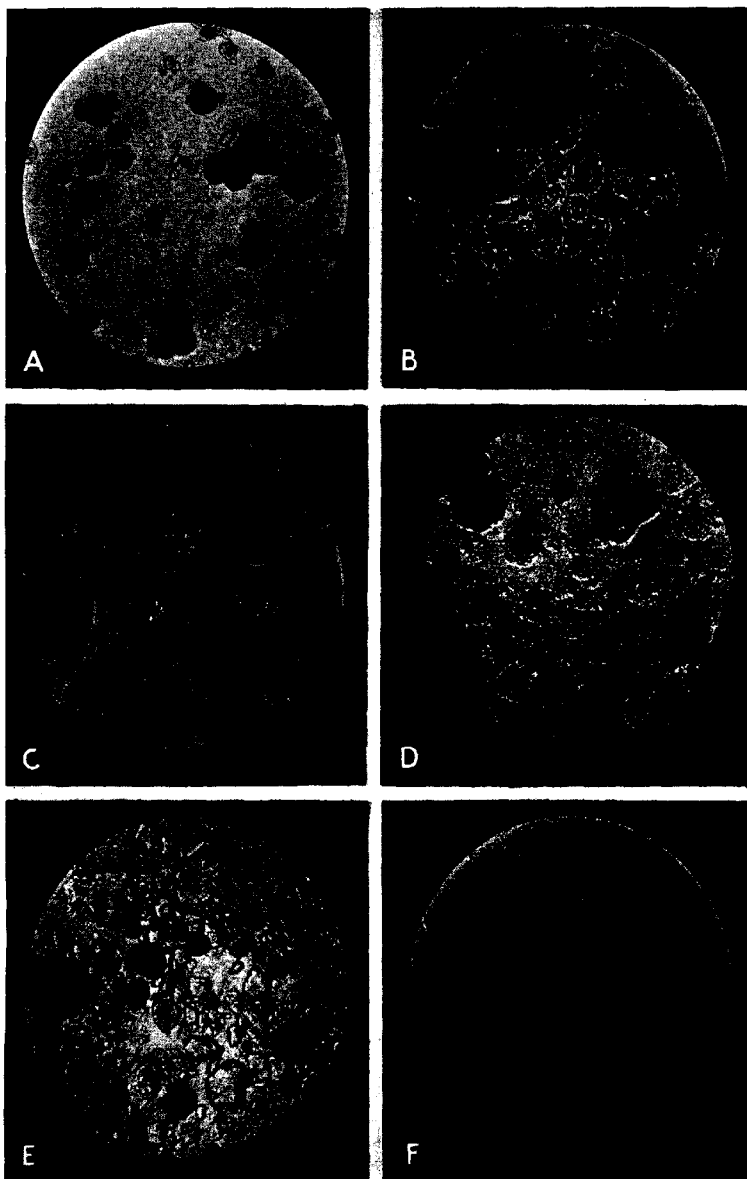


FIG. 26.—PHOTOMICROGRAPHS IDENTIFYING MALTED MILK AND ITS ALLIED PRODUCTS

## DESCRIPTION OF THE PHOTOMICROGRAPHS

## FIGURE 26

A.—SPRAY-DRIED WHOLE MILK: (a) MILK MASSES, (b) FAT GLOBULES; B.—SPRAY-DRIED MALT EXTRACT; C.—MECHANICAL MIXTURE: (a) SPRAY-DRIED MALT EXTRACT, (b) MILK MASSES, (c) FAT GLOBULES; D.—SPRAY-DRIED SKIM MILK; E.—MECHANICAL MIXTURE: (a) SPRAY-DRIED SKIM MILK, (b) SPRAY-DRIED MALT EXTRACT, (c) COCOA, (d) SUGAR; F.—DRUM-DRIED MALT EXTRACT.

A represents a spray-dried whole milk. The large particles represent aggregates of globular milk masses having a stippled surface (a). The fat appears as droplets (b).

B represents a spray-dried malt extract having the appearance of aggregates of droplets enclosed in spherical masses.

C represents a product made by mixing the spray-dried whole milk and the spray-dried malt extract, shown in A and B, in the proportion necessary to give the approximate composition of malted milk. The globular stippled milk masses (b) and the malt extract masses (a) are easily recognized; (c) fat globules.

D represents a spray-dried skim milk that might be confused with the spray-dried malt extract (B) because the structure of the spherical masses is similar. A comparison of the 2 pictures, however, will show that the droplets in the malt extract masses are larger than those appearing in the milk masses.

E represents a product purchased on the market and represented to contain malt, skim milk, whole milk, cocoa, and sugar. Examination shows malt extract (b), dried skim milk (a), cocoa (c), and sugar (d) present. Whole milk is absent. The cocoa consists of brown amorphous particles, easily discernible under the microscope. The highly refractive, irregular fragments of sugar cannot be mistaken.

F represents a drum-dried malt extract. It consists of clear, highly refractive fragments closely resembling broken glass.

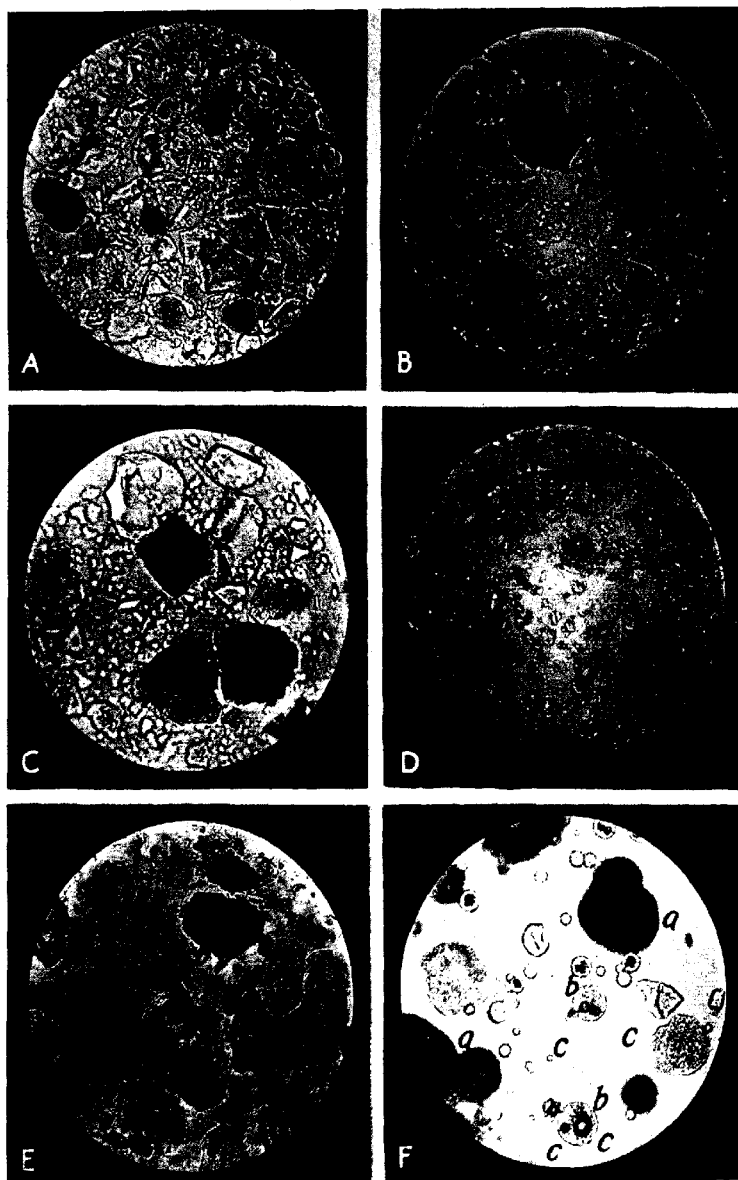


FIG. 27.—PHOTOMICROGRAPHS IDENTIFYING MALTED MILK AND ITS ALLIED PRODUCTS

## DESCRIPTION OF THE PHOTOMICROGRAPHS

## FIGURE 27

A.—MECHANICAL MIXTURE: (a) MILK MASSES, (b) SPRAY-DRIED MALT EXTRACT, (c) COCOA, (d) SUGAR; B.—MALTED MILK; C.—SWEET CHOCOLATE FLAVOR MALTED MILK; (a) MALTED MILK MASSES, (b) SUGAR; D.—MECHANICAL MIXTURE, (a) SPRAY-DRIED SKIM MILK, (b) SPRAY-DRIED MALT EXTRACT, (c) COCOA, (d) SUGAR; E.—MECHANICAL MIXTURE: (a) MALTED MILK MASSES, (b) COCOA, (c) SUGAR; F.—PRODUCT PREPARED FROM MALT INFUSION AND MILK BY SPRAY DRYING; (a) MILK MASSES, (b) MALT EXTRACT MASSES, (c) FAT GLOBULES.

A represents a mechanical mixture of dried whole milk (A, Fig. 26), dried malt extract (B, Fig. 26), cocoa and sugar. The milk (a) and malt extract (b) masses and the sugar particles (d) are readily recognizable. A mass of cocoa appears near the center of the picture (c).

B is characteristic of the genuine malted milks of the market. This picture cannot be mistaken for any product of similar composition. The malt extract solids and the milk solids are incorporated into homogeneous irregular fragments having a stippled surface.

C represents a "sweet-chocolate-flavor malted milk" purchased on the market, prepared by simultaneously evaporating in vacuo milk, malt infusion, cocoa and sugar. It is easy to recognize the characteristic malted milk masses (a), shown in the picture immediately preceding. They are slightly thicker and appear darker in the picture because the cocoa is intimately associated with them.

D represents a mechanical mixture of spray-dried skim milk (D, Fig. 26), spray-dried malt extract (B, Fig. 26), cocoa and sugar. No trouble is experienced in identifying these materials.

E represents a mechanical mixture of malted milk (B), cocoa, and sugar. Examination shows that malted milk (a) is present.

F represents a product found on the market under the label "malted milk." It shows none of the characteristics found in genuine malted milk, as is readily seen by a comparison with B. Individual milk masses (a), fat globules (c), and malt extract masses (b) closely resembling the spray-dried products (A and B, respectively, Fig. 26) predominate. Some particles show the stippled surface of genuine malted milk, but they are spherical instead of angular. A comparison of this picture with the picture showing the mechanical mixture of spray-dried whole milk and spray-dried malt extract (C, Fig. 26) shows a striking similarity.

maximum of 25. When the square root is not a whole number, sample one extra case. Select the cases to include each churn or batch mark when so marked. With less than 5 cases sample all, taking 5 prints as a minimum. Remove the wrapper and transfer each print to a separate sample container. (With prints of one pound or over the print may be quartered and two alternate quarters placed in the same container as the sample.) With 8 oz and 4 oz prints, take the whole print as the sample.

The above directions provide a minimum sampling, to be increased if the object of the examination demands.

(c) *Sample containers.*—Use a glass jar, preferably with glass top, of such type as will prevent loss of moisture by evaporation or entrance of  $H_2O$  into the jar. Tops containing a liner of any material should not be used.

## 76

## PREPARATION OF SAMPLE—OFFICIAL

Soften the entire sample in a closed vessel at as low a temp. as possible. Shake vigorously until a perfectly homogeneous, semi-solid mass is obtained. Weigh the portions for analysis at once. If the sample is kept for any length of time, soften and shake it until semi-solid before withdrawing portions for analysis.

## 77

*Mechanical Stirrer Method*<sup>27</sup>—Tentative

Soften the sample, 250–500 g, in a closed vessel, to such an extent that on stirring for 2–3 min. the product will reach a temp. of 31–34°. Stir with a malted milk stirrer for 2–3 min. with an up-and-down movement of the stirring device, at the same time slowly moving the vessel horizontally so that the stirrer reaches all parts of the sample. The final temp. must be 31–34°. If the temp. is below 31°, continue the softening and stirring until this temp. is reached. A temp. above 34° usually indicates that the sample has been softened too much, and is likely to separate. In this case, cool the sample until solid and repeat the softening and mixing. Weigh the portion for analysis within 3–4 hours if the room temp. is approximately 25°, or within 30 min. if the room temp. is 28° or above. Do not permit the butter to cool below 23° before weighing the portions for analysis.

## 78

## MOISTURE—OFFICIAL

Weigh accurately 1.5–2.5 g of the sample into a flat-bottomed dish having a surface of at least 20 sq. cm, dry at the temp. of boiling  $H_2O$ , and weigh at hourly intervals until the weight becomes constant. The use of clean, dry sand or asbestos is admissible.

MOISTURE, FAT, AND SALT<sup>28</sup>—TENTATIVE

## 79

## APPARATUS

*Gooch crucible.*—Prepare a Gooch crucible of about 30 cc capacity with a 0.1 g pad of asbestos and place thereon 20 g of R. R. alundum, 90-mesh. (This is crystalline alumina especially prepared for C determinations.) After use, again prepare the crucible for further use by igniting in a muffle, washing with  $H_2O$ , and drying at 100–105°.

## 80

## DETERMINATION

Weigh accurately in the weighed, specially prepared Gooch crucible 1.0–1.5 g of the prepared sample, 76, dry for 2 hours at 100–105°, cool, weigh, and calculate the loss in weight as moisture. Extract the fat from the dried sample by placing the Gooch crucible in a closed-system extraction apparatus and extracting for 30–40

min. with  $\text{CCl}_4$ . Adjust the heat so that the solvent drops into the crucible at the same rate as the crucible drains and keep the crucible nearly full of the solvent. When the extraction is complete, remove most of the solvent remaining in the crucible by applying suction for a few seconds. Dry the crucible for 30 min. at 100–105°, cool, weigh, and calculate the percentage of non-fat solids. Calculate the percentage of fat by subtracting the sum of the percentages of moisture and non-fat solids from 100.

To determine the salt, wash it out of the non-fat solids with  $\text{H}_2\text{O}$  and titrate the aqueous soln with standard  $\text{AgNO}_3$  soln, using  $\text{K}_2\text{CrO}_4$  indicator.

#### FAT

81

##### *Indirect Method—Official*

Take up the dry butter, obtained in the moisture determination in which no absorbent was used, in absolute ether or in petroleum ether; transfer to a weighed Gooch crucible, with the aid of a wash bottle filled with the solvent; and wash until free from fat. Dry the crucible and its contents at the temp. of boiling  $\text{H}_2\text{O}$  until the weight is constant and determine the fat by difference.

82

##### *Direct Method—Official*

From the dry butter, obtained in the determination of moisture either with or without the use of an absorbent, extract the fat with anhydrous, alcohol-free ether, or with petroleum ether (b.p. below 65°), receiving the soln in a weighed flask. Evaporate the ether, dry the extract at the temp. of boiling  $\text{H}_2\text{O}$ , and weigh at hourly intervals until the weight is constant.

83

#### CASEIN, ASH, AND SALT—OFFICIAL

Cover the crucible containing the residue from the fat determination by the indirect method, 81, and heat, gently at first; then raise the temp. gradually to just below redness. The cover may then be removed and heating continued until the contents of the crucible are white. The loss in weight represents casein, and the residue in the crucible, mineral matter. Dissolve this mineral matter in  $\text{H}_2\text{O}$  slightly acidified with  $\text{HNO}_3$  and determine Cl, either gravimetrically as directed under XII, 35, or volumetrically as directed under XII, 37, and calculate the NaCl.

84

#### SALT—OFFICIAL

Weigh in a counterpoised beaker 5–10 g of the sample; add about 20 cc of hot  $\text{H}_2\text{O}$ ; and after the butter has melted transfer the whole to a separatory funnel. Insert the stopper and shake for a few moments. Let stand until all the fat has collected on the top of the  $\text{H}_2\text{O}$ ; then draw off the  $\text{H}_2\text{O}$  into a flask, being careful to let none of the fat globules pass. Again add hot  $\text{H}_2\text{O}$ , rinsing the beaker, and repeat the extraction 10 to 15 times, using 10–20 cc of  $\text{H}_2\text{O}$  each time. The washings will contain all but a mere trace of the NaCl originally present in the butter. Determine the quantity in the whole or in an aliquot of the liquid by titration with standard  $\text{AgNO}_3$  soln, using  $\text{K}_2\text{CrO}_4$  indicator.

85

#### EXAMINATION OF FAT—OFFICIAL

Melt the butter and keep in a dry place at about 60° for 2–3 hours, or until the  $\text{H}_2\text{O}$  and curd have entirely separated. Filter the clear, supernatant fat thru a dry filter paper in a hot water funnel or in an oven at about 60°. If the filtered liquid fat is not perfectly clear, refilter. Determine physical and chemical constants as directed under XXXI.

86

**PRESERVATIVES—OFFICIAL**

Proceed as directed under 29 and under XXXII.

87

**COLORING MATTERS—OFFICIAL**

Pour about 2 g of the filtered fat, dissolved in ether, into each of 2 test tubes. Into one of the tubes pour 1–2 cc of HCl (1+2) and into the other about the same volume of 10% NaOH soln. Shake the tubes well and allow to stand. In the presence of azo dyes the test tube to which the acid has been added will show a pink to wine-red coloration, while the alkaline soln in the other tube will show no color. If, on the other hand, annatto or other vegetable color is present, the alkaline soln will be colored yellow, while no color will be apparent in the acid soln.

*General test.*—Proceed as directed under XXI, particularly 3 and 16(b), for the detection of oil-soluble coal tar dyes and annatto.

88

**MICROSCOPIC EXAMINATION—OFFICIAL**

(a) Place on a slide a small portion of the fresh unmelted sample taken from the inside of the mass, add a drop of pure olive oil, apply a cover-glass with gentle pressure, and examine with a magnification of 120–150 diameters for crystals of lard, etc. Examine another portion of the sample with polarized light and selenite plate without the use of oil. Pure fresh butter will show neither crystals nor a part-colored field with selenite. Renovated butter or other fats melted and cooled and mixed with butter will usually present crystals and variegated colors with the selenite plate.

(b) For further microscopic study dissolve in a test tube 3–4 cc of the fat in 15 cc of ether. Close the tube with a loose plug of cotton wool and allow to stand 12–24 hours at 20–25°. When crystals form at the bottom of the tube, remove with a pipet, glass rod, or tube; place on a slide, cover, and examine under a microscope. The crystals formed by later deposits may be examined in a similar way. Compare with crystals obtained in the same way from samples of known purity.

**RENOVATED BUTTER<sup>24</sup> AND OLEOMARGARINE**

89

*I. Foam Test—Tentative*

Heat 2–3 g of the sample in either a spoon or a dish over a small flame. True butter will foam copiously, whereas process butter will bump and sputter like hot grease, with little or no foaming. Oleomargarine behaves like process butter, but chemical tests will determine whether the sample is oleomargarine or butter.

90

*II. Melted Fat Test—Tentative*

Melt 50–100 g of butter or renovated butter at 50°. The curd from butter will settle, leaving a clear supernatant fat; in the case of renovated butter, the supernatant fat remains more or less turbid.

**CHEESE**

91

**SELECTION AND PREPARATION OF SAMPLE—OFFICIAL**

When the cheese can be cut, take a narrow, wedge-shaped segment reaching from the outer edge to the center of the cheese. Cut this into strips and pass 3 times thru a sausage machine. When the cheese cannot be cut, take the sample with a cheese trier. If only one plug can be obtained, take it perpendicularly to the surface of the cheese at a point  $\frac{1}{2}$  the distance from the edge to the center and extending either entirely or half way thru it. When possible draw 3 plugs, 1 from the center, 1 from a point near the outer edge, and 1 from a point half-way between the other two. For

inspection purposes reject the rind, but for investigations requiring the absolute quantity of fat in the cheese include the rind in the sample. Grind the plugs in a sausage machine (the preferable method) or cut them very finely and mix thoroly.

92

**MOISTURE<sup>a</sup>—OFFICIAL**

Weigh 2-3 g of the prepared sample, 91, into a previously weighed metal dish (diameter about 55 mm, height about 15 mm, provided with a slip-in inverted cover fitting tightly on the inside), cover tightly, and reweigh. In the case of soft cheese and process cheese of high moisture content, weigh 1-2 g and partially dry on a steam bath. Dry the cheese in the loosely covered dish, placed in direct contact with the metal shelf of the oven, to constant weight (approximately 4 hours) under a pressure not to exceed 100 mm (4 inches) of Hg at the temp. of boiling H<sub>2</sub>O. During the drying admit into the oven a slow current of air (about 2 bubbles per second) dried by passing thru H<sub>2</sub>SO<sub>4</sub>. Discontinue the action of the vacuum pump and carefully re-admit air into the oven. Press the cover tightly into the dish, remove the dish from the oven, cool, and weigh. Express the loss in weight as moisture.

93

**ASH<sup>a</sup>—TENTATIVE**

Weigh into a Pt dish 3-5 g of the prepared sample, place on the steam bath, and dry for approximately 1 hour. (If the cheese is rich in fat, a small amount of absorbent cotton may be placed in the dish.) Ignite cautiously to avoid spattering and remove the burner while the fat is burning. When the flame has died out, complete the ignition in a muffle at dull redness, not exceeding 550°.

94

**TOTAL CHLORIDES<sup>a</sup>—TENTATIVE**

Weigh accurately approximately 3 g of cheese into a 300 cc Erlenmeyer flask and add 25 cc of 0.1 N AgNO<sub>3</sub>, which is more than enough to combine with all the Cl. Add 10 cc of halogen-free HNO<sub>3</sub> and 50 cc of H<sub>2</sub>O and boil. As the soln boils add approximately 15 cc of 5% KMnO<sub>4</sub> soln in 5 cc portions. (The soln becomes yellowish and clear.) Dilute the soln to 200 cc in a graduated flask and filter. Titrate the excess AgNO<sub>3</sub> in 100 cc of the clear filtrate with 0.1 N KSCN, using 2 cc of a saturated soln of ferric alum as indicator. Run a blank on the reagents used, following the same procedure, except to add sugar to destroy the excess of permanganate. Calculate the Cl found to NaCl and report as such.

95

**NITROGEN—OFFICIAL**

Determine N in an accurately weighed portion (about 2 g) of the prepared sample as directed under II, 21, 23, or 25. The percentage of N $\times$ 6.38 = the percentage of N compounds.

96

**ACIDITY—OFFICIAL**

To 10 g of finely divided cheese add H<sub>2</sub>O at a temp. of 40° until the volume equals 105 cc, shake vigorously, and filter. Titrate 25 cc portions of the filtrate, as representing 2.5 g of the sample, with standard NaOH, preferably 0.1 N, using phenolphthalein indicator. Express the result in terms of lactic acid. 1 cc of 0.1 N NaOH soln = 0.0090 g of lactic acid.

97

**COLORING MATTERS—TENTATIVE**

Extract 25-50 g of the prepared sample, 91, with ether, remove the ether by evaporation, and proceed as directed under XXI, 3 or 15.

98

**FAT—OFFICIAL**

Rub up, by means of a glass rod, 1 g of the prepared sample with 9 cc of H<sub>2</sub>O and 1 cc of NH<sub>4</sub>OH in a narrow 100-125 cc beaker. Digest the mixture at a low heat



until the casein is well softened; neutralize with HCl, using litmus as an indicator; and add 10 cc more of HCl. Add about 0.5 g of sand previously digested with HCl to prevent bumping and boil gently for 5 min., keeping the beaker covered with a watch-glass. Cool the soln, transfer to a Röhrig tube or a similar apparatus, rinse the beaker with 25 cc of ether, and transfer the ether rinsings to the Röhrig tube, shaking thoroly. Add 25 cc of petroleum ether (b.p. below 65°), shake thoroly, and let the mixture separate. Proceed from this point as directed under 19, beginning with "Draw off as much as possible of the ether-fat soln."

99

**EXAMINATION OF FAT—OFFICIAL**

(a) *Alkaline extraction*.—Treat about 300 g of the cheese, cut into fragments the size of a pea, with 700 cc of 5% KOH soln at 20° in a large, wide-necked flask, shaking vigorously to dissolve the casein. In 5–10 min. the casein will be dissolved, and the fat will rise to the surface in lumps. Collect the lumps of fat into as large a mass as possible by shaking gently. Pour cold H<sub>2</sub>O into the flask until the fat is driven up into the neck and remove it by suitable means. Wash the fat thus obtained with just sufficient H<sub>2</sub>O to remove the residue of the alkali which it may contain. The fat is not perceptibly attacked by the alkali in this treatment, is practically all separated in a short time, and is then easily prepared for chemical analysis by filtering and drying as directed under 85. Examine the fat as directed under XXXI.

(b) *Acid extraction*.—Pass the cheese thru a grinding machine; transfer to a large flask; and cover with warm H<sub>2</sub>O, using 1 cc for every gram of cheese. Shake thoroly and add H<sub>2</sub>SO<sub>4</sub> slowly and in small quantities, shaking after each addition of acid. The total quantity of acid used should be the same as the quantity of H<sub>2</sub>O employed. Remove the fat, which separates after standing a few min., by means of a separatory funnel; wash free from acid; filter; and dry as directed under 85. Examine the fat as directed under XXXI.

**TARTARIC ACID<sup>25</sup>**

100

*Qualitative Test—Tentative*

To 5 g of the ground cheese, add 40 cc of H<sub>2</sub>O at a temp. of about 50° and shake until the cheese is thoroly broken up. Add 3 cc of a 1% H<sub>2</sub>SO<sub>4</sub> soln and shake vigorously; then add 2 cc of a 20% soln of phosphotungstic acid and again shake vigorously. Let stand for 5 min. and filter. To 25 cc of the filtrate add sufficient saturated Ba(OH)<sub>2</sub> soln to make alkaline and 25 cc of 95% (by volume) alcohol, shake vigorously, and allow to settle. Filter thru a Büchner funnel, using light suction, and wash the residue on the filter several times with H<sub>2</sub>O. Transfer a portion of the paste to a small evaporating dish and dry on the steam bath. Add a few cc of H<sub>2</sub>SO<sub>4</sub> and a few crystals of resorcin, and heat slowly. If tartaric acid is present, there is produced a rose-red color that is slowly discharged on dilution with H<sub>2</sub>O.

*Quantitative Method<sup>22</sup>—Official*

101

**REAGENTS**

(a) *Potassium chloride wash soln*.—Dissolve 15 g of KCl in 100 cc of H<sub>2</sub>O and add 20 cc of 95% (by volume) alcohol.

(b) *Tartaric acid soln*.—Dissolve 1.5 g of pure tartaric acid in previously boiled and cooled H<sub>2</sub>O and dilute to 100 cc at 20°. Titrate with 0.1 N NaOH soln to determine the quantity of tartaric acid in 10 cc of the soln.

102

**DETERMINATION**

Weigh 25 g of the ground cheese into a 500 cc wide-mouthed salt bottle and add, 25 cc at a time, 100 cc of H<sub>2</sub>O at a temp. of 50–60°, shaking vigorously after each

addition. If necessary, continue the shaking until the cheese is thoroly broken up. Then add 25 cc of 2% Na oxalate soln and shake vigorously for 1 min. Add 100 cc of 2% HCl soln, 25 cc at a time, shaking vigorously after each addition. Add 50 g of powdered KCl, and shake for 5 min. To avoid churning, keep the mixture warm (at about 50°) during the shaking. Transfer the contents of the bottle, with the aid of H<sub>2</sub>O, to a 300 cc volumetric flask, cool to 20°, and make up to the mark with H<sub>2</sub>O. Mix thoroly; let stand for 10 min., with occasional shaking, and filter thru a dry folded filter, discarding the first few cc of the filtrate. Disregard any opalescence and transfer 200 cc of the filtrate to a 250 cc volumetric flask. Neutralize with 1 *N* NaOH soln, using phenolphthalein indicator, and then add 5.2 cc in excess. Make up to the mark with H<sub>2</sub>O, mix thoroly, let stand for a few min., and filter thru a dry folded filter, discarding the first few cc of the filtrate. To 100 cc of the filtrate in a 250 cc beaker add, with constant stirring, 10 cc of the tartaric acid soln, 2 cc of glacial acetic acid, and 23 cc of 95% (by volume) alcohol. Cool in an ice bath, stir vigorously until the cream of tartar begins to crystallize, and let stand in a refrigerator overnight. Prepare a Gooch crucible, having a removable disk, with a pad of asbestos about 10 mm thick. Decant most of the liquid thru this filter, wash the precipitate into the crucible with the KCl wash soln, and wash the beaker and precipitate 3 times, using 20-30 cc of the wash soln in all. Place the asbestos and precipitate in the beaker in which the precipitation was made and wash the crucible with about 50 cc of hot H<sub>2</sub>O. Heat the soln to boiling and titrate the hot soln with 0.1 *N* NaOH soln, using phenolphthalein indicator. Calculate the percentage of tartaric acid in the cheese by means of the formula:

$$X = 14.26[0.015(B + 1.5) - A], \text{ in which}$$

*A* = g of tartaric acid in 10 cc of the tartaric acid soln reagent; and

*B* = cc of 0.1 *N* NaOH soln required for the titration.

In the factor 14.26 the concentration caused by the insoluble solids of cheese of average composition is also taken into consideration.

#### CITRIC ACID\*

##### 103

##### *Qualitative Test—Tentative*

To 10 g of the ground cheese, add 20 cc of H<sub>2</sub>O at a temp. of about 50° and shake vigorously until the cheese is thoroly broken up. Add 20 cc of H<sub>2</sub>SO<sub>4</sub> (1+1) and 2 cc of a 20% soln of phosphotungstic acid, and shake vigorously. Let stand for 5 min. and filter. To 20 cc of the filtrate add 10 cc of Br water and 5 cc of KBr soln (15 g in 40 cc of H<sub>2</sub>O) and proceed with the oxidation as directed in the quantitative determination. Add sufficient FeSO<sub>4</sub> soln to dissolve the precipitated MnO<sub>2</sub>. If citric acid is present, a heavy white precipitate that settles rapidly is formed.

##### 104

##### *Quantitative Method<sup>21</sup>—Official*

Weigh 25 g of the ground cheese into a 500 cc wide-mouthed salt bottle, and add, 25 cc at a time, 100 cc of H<sub>2</sub>O at a temp. of 50-60°, shaking vigorously after each addition. If necessary, continue the shaking until the cheese is thoroly broken up. Then add 25 cc of 2% Na oxalate soln and shake vigorously for 1 min. Add 100 cc of 1% H<sub>2</sub>SO<sub>4</sub> soln, 25 cc at a time, shaking vigorously after each addition. Add 3 cc of 20% phosphotungstic acid soln and shake; then add 25 g of powdered anhydrous Na<sub>2</sub>SO<sub>4</sub>, and shake for 5 min. To avoid churning, keep the mixture warm (at about 50°) during the shaking. Transfer the contents of the bottle with the aid of H<sub>2</sub>O to a 300 cc volumetric flask, cool to 20°, and make up to the mark with H<sub>2</sub>O. Mix thoroly, let stand for 10 min. with occasional shaking, and then filter thru a dry

folded filter, discarding the first few cc of the filtrate. Heat 200 cc of the filtrate to boiling and, while still hot, add 20 cc of  $\text{H}_2\text{SO}_4$  (1+1) and 2 cc of the phosphotungstic acid soln. Mix and allow to stand for 15 min. With the aid of  $\text{H}_2\text{O}$  transfer the mixture to a 250 cc volumetric flask, cool to  $20^\circ$ , make up to the mark with  $\text{H}_2\text{O}$ , and filter thru a dry folded filter. Transfer 100 cc of the clear filtrate to a 500 cc Erlenmeyer flask (about 0.3 g of washed and dried asbestos may be added). Add 10 cc of a freshly prepared saturated soln of Br water and 5 cc of KBr soln (5 g KBr in 40 cc of  $\text{H}_2\text{O}$ ), mix thoroly, and heat to  $48-50^\circ$ . Hold at this temp. for 5 min., add 25 cc of 5% permanganate soln, shake, and allow to stand for about 5 min. Cool the flask and contents to about  $8^\circ$ , add 40 cc of cold  $\text{FeSO}_4$  soln (20 g  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  in 100 cc of  $\text{H}_2\text{O}$  and 1 cc of  $\text{H}_2\text{SO}_4$ ), shake continuously for 5 min., and let the mixture stand overnight in the refrigerator. Decant the supernatant liquid thru a Gooch crucible, measure the volume of the filtrate (a) and wash the precipitate into the crucible with this filtrate. Wash the precipitate with 3 successive 20 cc portions of ice-cold  $\text{H}_2\text{SO}_4$  (1+100), sucking dry after each addition, and finally wash with 3 successive 20 cc portions of ice-cold  $\text{H}_2\text{O}$ . Dry the precipitate to constant weight over  $\text{H}_2\text{SO}_4$  in a vacuum desiccator, protecting the precipitate from strong light or, to save time, dry in a current of air passed thru  $\text{H}_2\text{SO}_4$ . Weigh, and remove the pentabromacetone by extracting first with 3 successive 20 cc portions of 95% (by volume) alcohol and then with 3 successive 20 cc portions of ether. Dry, and weigh the crucible. To the weight of the pentabromacetone add 0.004 g for each 100 cc of filtrate (a) to compensate for solubility of the pentabromacetone and multiply the result by 6.06 to obtain the percentage of anhydrous citric acid in the cheese. (In this factor consideration is taken of the concentration caused by the insoluble solids in 25 g of cheese. It is assumed that the solids of cheese are almost insoluble under the conditions maintained and that the average process cheese contains about 60% of solids. No allowance is made for variation in the salt or moisture content or for variation in the specific volume of the solids, as such variations do not appreciably affect the results.)

#### LACTOSE IN PROCESS CHEESE<sup>22</sup>

##### *Quantitative Method*

##### 105

Weigh 25 g of the ground cheese into a 500 cc wide-mouthed salt bottle, and add, in 25 cc portions, 100 cc of  $\text{H}_2\text{O}$  at a temp. of  $50-60^\circ$ , shaking vigorously after each addition. If necessary, continue the shaking until the cheese is thoroly broken up. Add 25 cc of 2% Na oxalate soln and shake vigorously for 1 min.; add 25 g of powdered  $\text{Na}_2\text{SO}_4$  and shake for 2 min.; add 10 cc of  $\text{H}_2\text{SO}_4$  (1+1) and shake; and then add 25 cc of 20% phosphotungstic acid soln and shake vigorously. Transfer the contents of the bottle to a 500 cc volumetric flask, cool immediately to  $20^\circ$ , and make to the mark with  $\text{H}_2\text{O}$ . Mix thoroly, allow to stand for 10 min. and then filter thru a dry folded filter. Transfer 150 cc of the filtrate to each of two 250 cc volumetric flasks, add 10% NaOH soln to one flask until the mixture is alkaline to litmus, then add 5 g of solid KCl, and mix thoroly. Cool to  $20^\circ$  and make to the mark with  $\text{H}_2\text{O}$ . Shake well, allow to stand for 10 min., and filter thru a dry folded filter. Determine the lactose in a 50 cc aliquot as directed in XXXIV, 57. Treat the contents of the other volumetric flask as directed in XXXIV, 23(c), using 10 cc of HCl, etc. Add 10% NaOH soln until alkaline to litmus, and add 5 g of solid KCl. Mix thoroly, cool to  $20^\circ$ , and make to the mark with  $\text{H}_2\text{O}$ . Let stand for 10 min. Filter if necessary thru a dry filter paper. Determine the lactose in a 50 cc aliquot as before. An agreement between the amount of cuprous oxide reduced before and after inversion establishes the absence of sucrose.

Since the insoluble material of cheese and the precipitated phosphotungstic acid occupies some space in the flask as originally made up, it is necessary to correct for this volume. From the average composition of cheese the volume of the precipitate was calculated to be 14 cc. To obtain the true amount of lactose present, multiply all results by the factor 0.97.

#### GUMS IN CHEESE\*

106

##### *Qualitative Test*

To 50 g of sample in a 400 cc beaker add 10 g of  $H_2SiO_3$  (not coarse silica). To prevent emulsification remove some of the fat by stirring the mixture with petroleum ether in generous portion, having the beaker surrounded by warm  $H_2O$ . Decant the ether and remove the last traces by warming and stirring. Add 50–80 cc of  $H_2O$ , a little at a time, with continuous rubbing, warming if necessary, until the mixture has the consistency of thin cream. Stir in slowly two volumes of alcohol and allow to stand 1 hour or longer. Filter with suction on a Büchner funnel. Return the precipitate to the beaker and re-extract several times with the warm petroleum ether to remove all fat. Again rub the precipitate into a thin cream paste with about 50 cc of  $H_2O$ , add two volumes of alcohol, and allow to stand 1 hour or longer. Filter, and wash with petroleum ether and then with alcohol in generous quantity. Remove the filtrate from the paper immediately, break up the precipitate, and dry at  $100^\circ$  for 2 hours, or as much longer as may be necessary to remove the formaldehyde formed during drying. Pulverize before completing the drying and add the precipitate to 150 cc continuously boiling  $H_2O$ ; filter rapidly. Make the filtrate acid with 3–5 drops of glacial acetic acid, add 5 cc of a 10% soln of  $CaCl_2$ , and boil a few minutes. Add 28%  $NH_4OH$  dropwise with stirring to point of maximum precipitation, as shown by a clear supernatant liquid on standing after boiling. Filter, and evaporate the filtrate, which should be clear, to 30 cc. To 15 cc add 15 cc of glacial acetic acid and 90 cc of alcohol. If no flocculent precipitate forms in 30 min., gum is absent. A flocculent precipitate may be gum, agar, or milk proteins. Cloudiness in the soln is not produced by gum. To the other 15 cc of filtrate add an equal volume of 28%  $NH_4OH$ . Filter, and add three volumes of alcohol to the filtrate. A flocculent precipitate may be gum or milk proteins. With 0.1% or more gum present the characteristic flocculent gum precipitate is formed. With less gum confusion with milk protein may arise. For verification, filter, wash well with alcohol, dry, and then dissolve the precipitate with boiling  $H_2O$ . Evaporate the soln to 10 cc, add an equal volume of  $HCl$  and a few crystals of phloroglucin, quickly bring to a boil, and boil 60–90 seconds. Gums give a red amber to deep red color, depending on the quantity present. Gums, excepting arabic, are precipitated in alkaline or acid soln by the alcohol. For arabic the solns must be acid. Karaya is best precipitated at a low acidity.

#### ICE CREAM (PLAIN)

107

##### PREPARATION OF SAMPLE—OFFICIAL

Allow the sample to soften at room temp. Owing to the fact that melted butter fat tends to separate out and rise to the surface, it is not advisable to soften the ice cream by heating on a water bath or over a flame. Mix thoroly by stirring with a spoon or egg beater or by pouring back and forth between beakers.

108

##### NITROGEN—OFFICIAL

Place 4–5 g of the sample in a digestion flask and proceed as directed under II, 25.

## FAT

109

*Röse-Gottlieb Method—Official*

Weigh 4 g of the thoroly mixed sample into a small dry beaker; add 3 cc of  $H_2O$ ; thoroly mix with a glass rod; and transfer to a Röhrig tube or a similar apparatus, washing out the remaining portion with the aid of an additional 3 cc of  $H_2O$ . Add 2 cc of  $NH_4OH$ , mix thoroly, and heat in a water bath at  $60^\circ$ . From this point proceed as directed under 19, beginning with "Add 10 cc of 95% alcohol and mix well."

110

## COLORING MATTERS—TENTATIVE

Curdle 150–200 g of the melted sample by adding an equal volume of  $H_2O$  and 10–20 cc of acetic acid. Heat the mixture to  $70$ – $80^\circ$ , stirring meanwhile, and allow to cool. Continue as directed under 30 and 87 and under XXI, particularly 3 and 21 for the detection of oil-soluble coal tar dyes and annatto.

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- <sup>23</sup> Ibid., 39.
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- <sup>26</sup> Ibid., **18**, 401 (1935).
- <sup>27</sup> Ibid., 402.
- <sup>28</sup> Ibid., **11**, 40 (1928).
- <sup>29</sup> Ibid., **17**, 66 (1934).
- <sup>30</sup> Ibid., **10**, 264 (1927); **11**, 41 (1928).
- <sup>31</sup> Ibid., **15**, 75 (1932); **17**, 66 (1934).
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- <sup>33</sup> Ibid., **18**, 79 (1935).

## XXIII. EGGS AND EGG PRODUCTS

1

### COLLECTION AND PREPARATION OF SAMPLE<sup>1</sup>—TENTATIVE

No simple rules can be made for the collection of a sample representative of the average of any particular lot of egg material as the conditions encountered may differ widely. Experienced judgment must be used in each instance. If large lots are under examination, it is best to draw a number of samples for separate analyses rather than to attempt to get one composite representative sample.

(a) *Liquid eggs*.—Secure representative container or containers. Mix the contents of a container thoroly and draw about 300 g. A long-handled dipper or ladle serves well. Keep the sample hermetically sealed in a jar in a cool place. Report odor and appearance.

(b) *Frozen eggs*.—Secure representative container or containers. Examine contents as to odor and appearance. The condition of the contents can be determined best by boring a hole to the center of a container with an auger and noting the odor as the auger is withdrawn. If impossible to secure individual containers, samples may consist of the composite of the borings from the contents of each container. Take the borings midway between the center and circumference of the top of the can from at least 3 widely separated parts and extend them as near the bottom of the can as possible. Collect about 300 g of the sample. Keep the sample hermetically sealed in a jar in a cool place and in a frozen state if possible. Before analyzing warm the sample in a bath held below 50° and mix well.

(c) *Dried eggs*.—Secure representative container or containers. For small packages, take entire parcel or parcels for the sample. For boxes and barrels, remove the top layer to a depth of about 6 inches with a flour scoop or other convenient instrument. Draw small quantities of sample totaling about 300–500 g from accessible parts of the container and place in a hermetically sealed jar. Report odor and appearance. Prepare the sample for analysis by mixing 3 times thru a domestic flour sifter to assure complete breaking up of lumps. Keep in a hermetically sealed jar in a cool place.

(d) *Flaked and drum dried eggs*.—Collect the sample as directed for powdered dried eggs. Report odor and appearance. Prepare albumin samples for analysis by grinding in a mill to pass entirely thru a 60-mesh sieve, and whole egg and yolk samples to pass entirely thru a 20-mesh sieve or as fine as is practicable. Keep in a hermetically sealed jar in a cool place.

### TOTAL SOLIDS

#### *I. Vacuum Method<sup>2</sup>—Official*

2

#### APPARATUS

*Vacuum oven*.—Connected with a pump to maintain a partial vacuum in the oven with a pressure equivalent to 25 mm or less of Hg and provided with a thermometer passing into the oven in such a way that the bulb is near the samples. Connect an H<sub>2</sub>SO<sub>4</sub> gas drying bottle with the oven for admitting dry air to release the vacuum.

3

#### DETERMINATION

(a) *Liquid eggs*.—Weigh accurately by difference by means of a weighing buret about 5 g of the sample 1(a) or (b) in a covered dish that previously has been dried at 98–100°, cooled in a desiccator, and weighed soon after attaining room temp.

Remove the cover and drive off most of the  $H_2O$  by heating on the steam bath. Replace the cover loosely and complete the drying in the vacuum oven as directed under (b).

(b) *Dried eggs*.—Weigh accurately about 2 g of the sample, 1(c), in a covered dish that previously has been dried at 98–100°, cooled in a desiccator, and weighed soon after attaining room temp. Loosen the cover (do not remove) and heat at 98–100° to constant weight (approximately 5 hours) in the vacuum oven. Admit dry air into the oven to bring to atmospheric pressure. Immediately tighten the cover on the dish, transfer to a desiccator containing a fresh efficient desiccant, and weigh soon after room temp. is attained. Report the weight of egg residue as total solids.

#### ORGANIC AND AMMONIACAL NITROGEN<sup>3</sup>—OFFICIAL

4

##### PREPARATION OF SAMPLE

(a) *Liquid eggs*.—Weigh 2–3 g of the well-mixed sample, 1(a) or (b), by difference into a 500 cc Kjeldahl flask.

(b) *Dried eggs*.—Transfer about 1 g of the sample, 1(c), accurately weighed, to a 500 cc Kjeldahl flask.

5

##### DETERMINATION

Determine N as directed under II, 21, or preferably 23 or 25. (Complete digestion of the sample is accomplished most rapidly by the Kjeldahl-Gunning-Arnold method.) Distil the  $NH_3$  into 30–50 cc of 0.1 N standard acid.

#### WATER-SOLUBLE NITROGEN AND CRUDE ALBUMIN NITROGEN<sup>4</sup>—OFFICIAL FOR LIQUID EGGS, TENTATIVE FOR DRIED EGGS

6

##### PREPARATION OF SOLUTION

(a) *Liquid eggs*.—Weigh accurately, by difference, into a 250 cc volumetric flask containing 150 cc of  $H_2O$ , approximately 10 g of the well-mixed sample, 1(a) or (b), and mix gently. Add 5 cc of 0.01 N acetic acid soln for each g of egg substance, fill to mark with  $H_2O$ , shake gently, and filter thru an 18½ cm folded filter, covering the filter with a watch-glass during filtration. If the filtrate is cloudy, allow the filtration to continue until drops of filtrate become clear, change the receiving container, return the cloudy filtrate to the filter, and proceed as directed under 7.

(b) *Dried eggs*.—From the sample, 1(c), transfer 1 g of whites, 3 g of whole eggs, or 5 g of yolks into an 8 ounce nursing bottle, add 50 cc of petroleum ether, mix gently, centrifuge, and decant the solvent. Repeat the treatment with petroleum ether. Place the bottle on its side, rolling it occasionally, until the residue is dry, and break up the dry residue with a glass rod having a flattened end. Add 100 cc of  $H_2O$  (slowly at first with gentle mixing until sample disintegrates), then add 5 cc of 0.01 N acetic acid soln for each g of egg substance and sufficient  $H_2O$  to make a total of exactly 200 cc of  $H_2O$  and acid. Mix gently and allow to stand 2 hours, continuing the mixing at intervals. Centrifuge, filter, and proceed as directed under 7.

7

##### DETERMINATION

(a) *Water-soluble nitrogen*.—Transfer 50 cc of the clear prepared filtrate into a 500 cc Kjeldahl flask, and determine N as directed under II, 25, using mercuric oxide. Calculate the N and report as percentage of water-soluble N.

(b) *Crude albumin nitrogen*.—Transfer 100 cc of the clear prepared filtrate to a 200 cc volumetric flask, add 15 cc of NaCl soln (28 g NaCl diluted to 300 cc), fill nearly to mark with 95% alcohol, and mix. Cool to room temp., fill to mark with 95% alcohol, shake, and allow to stand overnight. Filter, transfer 100 cc of the

filtrate to a 500 cc Kjeldahl flask, and determine N as directed under II, 25, using mercuric oxide. Calculate the percentage of N, subtract it from the percentage of water-soluble N, and report the difference as percentage of crude albumin N.

#### FAT BY ACID HYDROLYSIS<sup>a</sup>—TENTATIVE

8

##### PREPARATION OF SOLUTION

(a) *Liquid eggs*.—From the well-mixed sample, 1(a) or (b), weigh accurately by difference into a fat extraction tube (Mojonnier tube is convenient) approximately 2 g of yolks, or 3 g of whole eggs, or 5 g of whites. Add slowly with vigorous shaking 10 cc of HCl, set the tube into a water bath heated to 70°, bring to boiling, and continue the heating at boiling for 30 min., shaking the tube with care at 5 min. intervals. Remove the tube from the water bath, add H<sub>2</sub>O nearly to fill the lower bulb of the tube, and cool to room temp.

(b) *Dried eggs*.—Transfer 1 g of the well-mixed sample to the fat extraction tube; add slowly, washing down any egg particles adhering to the sides of the tube, 10 cc of HCl (4+1); and proceed as directed in (a).

9

##### DETERMINATION

To the extraction tube containing the sample treated as directed under 8, add 25 cc of ethyl ether, and mix. Add 25 cc of redistilled petroleum ether (b.p. below 60°), mix, and allow to stand until the ether layer is clear. Decant the clear ether layer into a weighed 125 cc beaker flask containing 2-3 small porcelain chips, and evaporate the ethers slowly on a water bath. Re-extract the liquid remaining in the tube twice more, using 15 cc of each ether and mixing after the addition of each ether. Allow to stand until the ether layer is clear, decant into the 125 cc beaker flask, and evaporate slowly on a water bath. Dry the beaker flask and contents at 100° to minimum weight (approximately 90 min.). Allow the flask to stand in the air until no further change in weight takes place (approximately 30 min.), and weigh. Correct this weight by a blank determination on the reagents. Report as percentage of fat by acid hydrolysis.

#### LIPIDS AND LIPOID PHOSPHORIC ACID (P<sub>2</sub>O<sub>5</sub>)<sup>a</sup>—TENTATIVE

10

##### REAGENTS

(a) *Mixed solvent*.—Equal volumes of CHCl<sub>3</sub> and absolute alcohol.

(b) *Alcoholic sodium hydroxide*.—Prepare a saturated soln free from carbonates by dissolving 100 g of NaOH in 100 cc of H<sub>2</sub>O. Allow the mixture to stand until clear, or filter thru a hard filter paper which has been soaked in alcohol (5 cc of the NaOH soln contains approximately 4 g of NaOH). Dissolve 50 cc of this soln in 900 cc of 95% alcohol and dilute with 95% alcohol to 1 liter.

11

##### PREPARATION OF SOLUTION

(a) *Liquid eggs*.—Weigh accurately by difference approximately 4 g of the well-mixed sample, (a) or (b), into a 100 cc volumetric flask, add very slowly (dropwise) from a pipet, 25 cc of the mixed solvent, shaking constantly until the proteins become coagulated and then thoroly broken up. Add 60-65 cc more of the solvent and allow to stand 1 hour, shaking at 5 min. intervals. Fill to the mark with the solvent, shake, and allow the mixture to stand until clear.

(b) *Dried eggs*.—Transfer 2 g of the well-mixed sample, 1(c), into a 100 cc volumetric flask, add 85-90 cc of the mixed solvent, and allow to stand 1 hour, mixing at 5 min. intervals. Proceed as directed in (a).



## 12

## DETERMINATION

(a) *Lipids*.—Transfer a 50 cc aliquot to a 150 cc beaker and evaporate the extract to dryness on a steam bath. (An electric fan or a gentle blast of dry air may be used to hasten evaporation.) Place the beaker into an oven at 100° for 5–10 min. to remove any remaining moisture. Dissolve the dry extract in 5–10 cc of  $\text{CHCl}_3$ , and filter the soln into a weighed 100 cc Pyrex beaker thru a pledget of cotton packed into the stem of a funnel, transferring all soluble extract from the bottom and sides of the beaker by means of  $\text{CHCl}_3$  from a wash bottle. Finally wash the funnel and stem tip. (The filtrate should be clear.) Evaporate the  $\text{CHCl}_3$  on a steam bath, and dry the beaker and contents in an oven at 100° to minimum weight (approximately 90 min.). Allow the beaker to stand in the air until no further change in weight takes place (approximately 30 min.), weigh, and report the percentage of lipids.

(b) *Lipoid phosphoric acid* ( $\text{P}_2\text{O}_5$ ).—Dissolve the dried lipoids in 2–3 cc of  $\text{CHCl}_3$ , add 10–20 cc of the alcoholic NaOH soln, evaporate to dryness on a steam bath, using care to avoid spattering, and place the beaker into an oven at 100° for 30 min. to remove any remaining moisture. Transfer the beaker while hot to an electric muffle heated to 500° (faint redness), and allow it to remain at that temp. for 1 hour. Cool, add a few drops of  $\text{H}_2\text{O}$ , break up the charge with a glass rod (flattened end), cover the beaker with a watch-glass, add slowly 5 cc of  $\text{HNO}_3$  (1+3), mix, remove the watch-glass, and filter, collecting the filtrate in a 300 or 500 cc Erlenmeyer flask. Thoroughly wash the charred material and filter paper with  $\text{H}_2\text{O}$  from a wash bottle.

In the prepared filtrate determine phosphoric acid ( $\text{P}_2\text{O}_5$ ) as directed under II, 12, using 20–50 cc of the molybdate soln. Report the percentage of lipid  $\text{P}_2\text{O}_5$  in the eggs.

## 13

## UNSAAPONIFIABLE MATTER—TENTATIVE

Extract the lipoids as directed under 12(a). Determine the unsaponifiable matter in the extracted lipoids by the official F.A.C. method (XXXI, 37).

TOTAL PHOSPHORIC ACID ( $\text{P}_2\text{O}_5$ )—OFFICIAL

## 14

## PREPARATION OF SOLUTION

(a) *Liquid eggs*.—From the well-mixed sample, 1(a) or (b), weigh accurately, by difference, into a 250 cc low-form Pyrex beaker, approximately 2 g of yolk, 4 g of whole eggs, or 10 g of whites. Add 20 cc of 10%  $\text{Na}_2\text{CO}_3$  soln and evaporate to dryness on an electric hot plate or overnight at 100–105°. Transfer the beaker while hot to an electric muffle heated to 500° (faint redness), and allow it to remain at this temp. for 1 hour. Cool, add a few drops of  $\text{H}_2\text{O}$ , break up the charge with a glass rod with a flattened end, and cover the beaker with a watch-glass. Then add slowly and with continuous stirring 10 cc of  $\text{HNO}_3$  (1+3) and filter, collecting the filtrate in a 300 or 500 cc Erlenmeyer flask. Thoroughly wash the charred material and filter with  $\text{H}_2\text{O}$  from a wash-bottle.

(b) *Dried eggs*.—Transfer 1 g of the well-mixed sample, 1(c), to a 150 cc low-form Pyrex beaker, add 20 cc of 10%  $\text{Na}_2\text{CO}_3$  soln, and proceed as directed under (a).

## 15

## DETERMINATION

In the prepared filtrate determine the  $\text{P}_2\text{O}_5$  as directed under 12(b) and II, 12, using 40–50 cc of the molybdate soln. Report as total  $\text{P}_2\text{O}_5$ .

CHLORINE<sup>9</sup>—OFFICIAL

16

## PREPARATION OF SOLUTION

(a) *Liquid eggs*.—From the well-mixed sample, 1(a) or (b), weigh accurately, by difference, approximately 4 g of yolk, 7 g of whole eggs, or 10 g of whites into a 150 cc low-form Pyrex beaker; add 20 cc of 10%  $\text{Na}_2\text{CO}_3$  soln, mix, and evaporate to dryness on an electric hot plate or overnight at 100°. Transfer the beaker while hot to an electric muffle heated to 500° (faint redness), and allow it to remain at that temp. for 1 hour. Cool, add a few drops of  $\text{H}_2\text{O}$ , and break up the charge with a glass rod. Add 50 cc of  $\text{H}_2\text{O}$ , cover the beaker with a watch-glass, add slowly 20 cc of  $\text{HNO}_3$  (1+3), mix, remove the watch-glass, and filter, collecting the filtrate in a 200 cc volumetric flask. Wash the charred material and filter thoroly with  $\text{H}_2\text{O}$  from a wash bottle, keeping the total volume of filtrate to 180 cc or less.

(b) *Dried eggs*.—From the well-mixed sample, 1(c), transfer to a 150 cc low-form Pyrex beaker, 2 g of whole eggs or yolks, or 1 g of whites, and proceed as directed under (a).

17

## DETERMINATION

Add 10 cc of 0.1 *N*  $\text{AgNO}_3$  soln to the prepared filtrate and proceed as directed under XII, 35.

DEXTRSE AND SUCROSE<sup>10</sup>—OFFICIAL, FIRST ACTION

18

## PREPARATION OF SOLUTION

(a) *Liquid eggs*.—Weigh accurately by difference approximately 25 g of the well-mixed sample, 1(a) or (b), into a 250 cc volumetric flask containing 1 g of  $\text{CaCO}_3$  and 50 cc of the 5%  $\text{NaCl}$  soln. Add with continuous mixing 130 cc of 95% alcohol. Allow to stand a few min. for gas bubbles to rise to the surface, cool to room temp., fill to the mark with  $\text{H}_2\text{O}$ , shake, and filter (18½ cm folded filter). Transfer 150 cc of the filtrate to a 250 cc beaker, evaporate to 20–30 cc to remove the alcohol, cool, wash with  $\text{H}_2\text{O}$  into a 100 cc volumetric flask, holding the volume to 80–90 cc; add dry powdered phosphotungstic acid in small quantities in slight excess to precipitate any protein, mix, let stand a few minutes for gas bubbles to rise to the surface, fill to the mark with  $\text{H}_2\text{O}$ , shake, and filter. To the filtrate, add in very small portions sufficient dry powdered  $\text{KCl}$  to precipitate any excess phosphotungstic acid, filter if necessary, and test the filtrate for complete precipitation.

(b) *Dried eggs*.—From the well-mixed sample, 1(c), transfer to a 250 cc volumetric flask containing 1 g of  $\text{CaCO}_3$  and 50 cc of 5%  $\text{NaCl}$  soln 2.5 g of whites, or 10 g of yolks or whole eggs, and allow to stand 1 hour, mixing at 5 min. intervals. Add with continuous mixing 130 cc of 95% alcohol, and proceed as directed under (a).

19

## DETERMINATION

*Reducing sugars direct*.—Transfer 25 cc of the prepared filtrate to a 400 cc beaker, and proceed as directed under XXXIV, 37. Report as percentage of dextrose.

*Reducing sugars invert*.—Transfer 50 cc of the prepared filtrate to a 100 cc volumetric flask, add 5 cc of  $\text{HCl}$ , and allow to stand overnight. Neutralize with  $\text{NaOH}$  soln, cool to room temp., and fill to the mark with  $\text{H}_2\text{O}$ . Transfer 50 cc (or less) to a 400 cc beaker, and proceed as directed under XXXIV, 37. Deduct the percentage of invert sugar obtained before inversion from that obtained after inversion, multiply the difference by 0.95, and report as percentage of sucrose.

GLYCEROL<sup>1</sup>*Qualitative Test—Tentative*

20

## REAGENTS

*Fuchsin sulfite soln.*—Dissolve 0.2 g of fuchsin in 120 cc of hot H<sub>2</sub>O, cool the soln, add a soln of 2 g of anhydrous sodium sulfite in 20 cc of H<sub>2</sub>O, following with 2 cc of HCl. Dilute the soln with H<sub>2</sub>O to 200 cc and allow the mixture to stand for 1 hour before using.

21

## DETECTION

Add 20 cc of ethyl alcohol to approximately 5 g of sample in an Erlenmeyer flask or beaker flask, shake vigorously, and filter thru a 12.5 cm fluted filter paper. Evaporate the filtrate rapidly until no odor of alcohol is perceptible, cool, and add 3–4 drops of H<sub>2</sub>O and then 10–15 cc of anhydrous ethyl ether. Mix the solns carefully, allow to separate, and pour off as much as possible of the ether layer, disregarding cloudiness in this layer. Shake well with two 10 cc portions of anhydrous ether, pouring off the ether carefully in each case. (The volume of aqueous soln should not be less than 0.4–0.5 cc.) Evaporate off the remaining ether on a steam bath and continue the evaporation until 0.1–0.2 cc of liquid remains. Cool, and add 15 cc of a mixture of equal volumes of absolute alcohol and CHCl<sub>3</sub>. Cool, shake, and allow the mixture to stand for 5 min. to permit crystallization of sugar. Shake, and filter thru a fluted filter paper into a 6×1 inch test tube (hard glass). Evaporate the filtrate rapidly (a small flame in front of a fan is convenient) until no odor of chloroform or alcohol is perceptible. Add several grams of powdered potassium sulfate and insert a stopper with a glass tube leading into 2 cc of H<sub>2</sub>O in a test tube immersed in ice H<sub>2</sub>O. Heat with a small flame until frothing ceases and the contents of the tube are liquid. Remove the receiver, add immediately 4–5 drops of the fuchsin sulfite reagent, and warm to room temp. In the presence of glycerol a strong pink color (due to acrolein) develops within 1 min. and becomes a deep violet within 5 min.

*Quantitative Method—Tentative*

(Not applicable in the presence of sugars.)

22

## REAGENTS

- (a) *Mercuric nitrate soln.*—Prepare as directed under XXII, 64.
- (b) *Diphenylamine indicator soln.*—Dissolve 1 g of diphenylamine in 100 cc of H<sub>2</sub>SO<sub>4</sub>.
- (c) *Phosphoric acid-sulfuric acid soln.*—Add 150 cc of H<sub>2</sub>SO<sub>4</sub> and 150 cc of sirupy phosphoric acid to 500 cc of H<sub>2</sub>O and dilute with H<sub>2</sub>O to 1 liter.
- (d) *Potassium dichromate soln.*—Prepare as directed under XXXIII, 71(a).
- (e) *Ferrous ammonium sulfate soln.*—Prepare as directed under XXXIII, 71(c).
- (f) *Basic lead acetate soln.*—Prepare as directed under XXXIV, 18(a).
- (g) *Thymol blue indicator soln.*—Dissolve 0.1 g of thymol blue in 21.55 cc of 0.01 N NaOH soln and dilute to 250 cc with H<sub>2</sub>O.

23

## DETERMINATION

Weigh by difference approximately 5 g of sample into a 100 cc volumetric flask containing 50–75 cc of H<sub>2</sub>O, mix well, add 2 cc of the mercuric nitrate soln, again mix well, and make up to the mark with H<sub>2</sub>O. Mix, and transfer the contents of the flask to an 8-oz. centrifuge bottle. Add 5 g of light magnesium carbonate, stopper,

and shake vigorously for several min. Centrifuge for 1-2 min., pour off the supernatant liquid thru a fluted filter, and transfer 75 cc to a centrifuge bottle. Add 50 cc of the basic lead acetate soln and then 50 cc of 2 *N* KOH soln, mix, and add a drop of the thymol blue indicator soln. If the surface of the liquid does not turn deep blue, add more alkali. Let stand for 5-10 min., centrifuge until clear and pour off the liquid into a 250 cc volumetric flask. Shake the residue with 20 cc of H<sub>2</sub>O, centrifuge, and add the washings to the flask. Add 2 drops of thymol blue to the soln and add H<sub>2</sub>SO<sub>4</sub> (1+1) until a distinct pink color is obtained. Make up to the mark with H<sub>2</sub>O, mix, and filter thru a dry filter paper. Transfer 200 cc of the filtrate to a 400 cc beaker, evaporate rapidly to 75 cc, and then finish the evaporation on a water bath or slow hot-plate to 35-40 cc. Transfer the liquid to a 50 cc volumetric flask and make up to volume with H<sub>2</sub>O. Transfer 25 cc to a 250 cc volumetric flask, and add 20 cc of the K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> soln and 25 cc of H<sub>2</sub>SO<sub>4</sub>. Run a blank, using 20 cc of the dichromate soln and 25 cc of H<sub>2</sub>O. Heat in a *boiling* water bath for exactly 20 min. Cool, dilute to volume, mix, and transfer some of the soln to a buret. Pipet 20 cc of the Fe(NH<sub>4</sub>)<sub>2</sub>(SO<sub>4</sub>)<sub>2</sub> soln into a beaker, add 100 cc of H<sub>2</sub>O, 15 cc of the phosphoric-sulfuric acid soln and exactly 3 drops of the diphenylamine soln, and titrate with the K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> soln. When the green color has changed to a blue-gray, add the K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> soln slowly, swirling after each drop. The end point is reached when the addition of 1 drop of the K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> soln changes the color to a deep violet. Subtract 0.05 cc from the reading to correct for oxidation of the indicator.

$$\text{Percentage glycerol} = \frac{100(a-b)}{W a}, \text{ in which}$$

$a$  = cc of K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> titrated in unknown soln,  
 $b$  = cc of K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> titrated in blank soln, and  
 $W$  = weight of sample in grams.

ACIDITY OF ETHER EXTRACT<sup>10</sup>—OFFICIAL

(Not applicable to egg white.)

## 24

## REAGENTS

(a) *Benzene*.—Use the best available quality of benzene. If it is not neutral, titrate 50 cc with the 0.05 *N* Na ethylate and correct subsequent results accordingly.

(b) *Sodium ethylate*.—0.05 *N*. Dissolve a piece of metallic Na, approximately 1 cc in volume, in 800 cc of absolute alcohol. Titrate 10 cc of 0.1 *N* HCl with this soln and add the calculated volume of absolute alcohol to make the soln 0.05 *N*. Ascertain the normality factor by titration against 0.1 *N* HCl on the day the soln is used.

## 25

## DETERMINATION

(a) *Dried eggs*.—Weigh in a tared Al dish about 63 mm in diameter 2 g of the powdered sample, 1(c), and dry at 55° under a pressure not exceeding 125 mm of Hg. Weigh to the third decimal place at the end of 2 hours and make further weighings at half-hour intervals until no further loss in weight occurs. Extract the dried residue with anhydrous ether, preferably in a Knorr apparatus. Carefully transfer the egg powder to a 12.5 cm hardened filter paper, fold the paper once, place it on a 15 cm qualitative filter paper, and roll the papers and contents into a cylinder that will fit snugly into the extraction tube, folding in one end of the cylinder to prevent loss of material. (An asbestos plug is not needed in the extraction tube, and if the extractor is working rapidly, 3 hours is sufficient to insure proper extraction.) Evaporate the ether from the extraction flask, dry the extract for 1

hour at 55° under a pressure not exceeding 125 mm, and weigh to the third decimal place. Dissolve the extract in 50 cc of benzene, add 3 to 4 drops of phenolphthalein indicator, and titrate with the Na ethylate soln. The end point is reached when the yellow color changes to orange. Express the result as the number of cc of 0.05 *N* Na ethylate required per g of ether extract.

(b) *Liquid eggs*.—Weigh to the third decimal place in a weighed Pb dish about 5 g of the sample, 1(a) or (b), and dry as directed under (a). Weigh after drying for about 5 hours and thereafter, at 1 hour intervals, until no further loss in weight occurs. To prepare the dried residue for extraction with ether, place the dish upon a 12.5 cm hardened filter paper, cut the sides of the dish thru at 4 equidistant points, and flatten down. Place another similar filter paper on top of the dish and its contents and roll the papers and dish into a cylinder that will fit snugly into the extractor, folding in one end of the cylinder to prevent any of the egg residue from dropping into the extraction flask. Proceed as directed under (a).

#### AMMONIA NITROGEN<sup>12</sup>—TENTATIVE

(For liquid eggs.)

#### 26

##### APPARATUS

The apparatus consists of a train, items (a), (b), (c), and (d), each provided with a two-holed rubber stopper connected with glass tubing of suitable shape and length to permit proper passage of air, which is supplied by a pump with a pressure of 10 lbs. per sq. in. Compensation for the pulsations of the pump to assure delivery of a steady pressure is accomplished by placing a tank of sufficient size between the pump and bottle (a). Suction may be used to draw air thru the train but pressure is preferred.

(a) *Wash bottle*.—Contains  $\text{H}_2\text{SO}_4$  (about 35%) for removal of ammonia from the air supply. The inlet tube is provided with a stopcock to regulate the air supply.

(b) *Trap*.—To prevent mechanical transfer of  $\text{H}_2\text{SO}_4$  into (c).

(c) *Aerating cylinder*.—About 50 mm in diameter and 350 mm high. The inlet tube extends to within  $\frac{1}{2}$  in. of the bottom. The outlet tube is provided with a trap containing cotton or glass wool to prevent liquid from being carried over mechanically.

(d) *Bottle*.—Wide-mouthed, 8 oz. The end of the inlet tube terminates in a small bulb punctured with a few small holes to expedite ammonia absorption.

#### 27

##### DETERMINATION

Weigh approximately 25 g of sample, 1(a) or (b), in a convenient container. Pour as much as possible of this material into the aeration cylinder (c) and transfer the remainder by means of four 25 cc portions of ammonia-free  $\text{H}_2\text{O}$ , stirring each time with a rubber policeman to remove any egg adhering to the sides of the weighing vessel. Add 75 cc of alcohol, mix well, and let stand for 15 min. Add approximately 1 g of NaF, 5 cc of a 20% soln of  $\text{Na}_2\text{CO}_3$ , and 1 cc of kerosene. If necessary at this point the determination may stand overnight before the addition of the  $\text{Na}_2\text{CO}_3$ .

Connect the train and aerate thru 10 cc of 0.02 *N*  $\text{H}_2\text{SO}_4$  (if the sample has a bad odor, it may be necessary to use more than 10 cc of 0.02 *N*  $\text{H}_2\text{SO}_4$ ), 2 drops of methyl red indicator (saturated soln in 95% alcohol), and about 75 cc of ammonia-free  $\text{H}_2\text{O}$  in the receiving bottle (d).

Use as rapid a current of air as possible without splashing the egg soln into the trap following cylinder (c). Determine the time to aerate as follows: In a duplicate

train measure 20 cc of  $\text{NH}_4\text{Cl}$  or  $(\text{NH}_4)_2\text{SO}_4$  soln (about 5% stronger than 0.02 N) into the aeration cylinder (c) and 20 cc of 0.02 N  $\text{H}_2\text{SO}_4$ , 75 cc of  $\text{H}_2\text{O}$ , and several drops of methyl red indicator into the receiving bottle (d). Aerate until the soln in the receiving bottle changes color. The sample should be aerated for 30 min. longer than the time required for this color change.

Titrate the excess of acid in cylinder (d) with 0.02 N NaOH (free from  $\text{CO}_2$ ). Express results as mg of ammonia nitrogen per 100 g of sample on the wet basis. Correct the results for a blank determination on the apparatus and reagents.

28

## EXTRACTION AND IDENTIFICATION OF ADDED COLOR

Determine as directed under **XX, 72**.

## SELECTED REFERENCES

- <sup>1</sup> J. Assoc. Official Agr. Chem., **8**, 599 (1925); **9**, 56 (1926).
- <sup>2</sup> Ibid., **8**, 600 (1925); **9**, 56, 354 (1926); **14**, 85, 395 (1931).
- <sup>3</sup> Ibid., **8**, 601 (1925); **9**, 57 (1926).
- <sup>4</sup> Ibid., **75**, 344 (1932); **16**, 74 (1933); **18**, 80 (1935).
- <sup>5</sup> Ibid., **8**, 601 (1925); **9**, 58 (1926); **16**, 73, 298 (1933).
- <sup>6</sup> Ibid., **7**, 91 (1923); **8**, 602 (1925); **9**, 58 (1926); **16**, 73 (1933).
- <sup>7</sup> Ibid., **9**, 122 (1926); **12**, 56, 347 (1929).
- <sup>8</sup> Ibid., **14**, 85, 416 (1931); **16**, 73, 298 (1933); **18**, 80 (1935).
- <sup>9</sup> Ibid., **14**, 85 (1931); **16**, 74, 298 (1933); **18**, 80 (1935).
- <sup>10</sup> Ibid., **14**, 397 (1931); **16**, 74, 305 (1933).
- <sup>11</sup> Ibid., **16**, 74 (1933).
- <sup>12</sup> Ibid., **10**, 50, 411 (1927); U. S. Dept. Agr. Bull. 846, p. 89.
- <sup>13</sup> Ibid., **6**, 7 (1922); U. S. Dept. Agr. Bull. 846.

## XXIV. FISH AND OTHER MARINE PRODUCTS\*

\* See note at bottom of p. xvii.

## XXV. FLAVORING EXTRACTS

### VANILLA EXTRACT AND ITS SUBSTITUTES

1

#### SPECIFIC GRAVITY—OFFICIAL

Determine the specific gravity at 20/20° by means of a pycnometer as directed under XIV, 3.

2

#### ALCOHOL—OFFICIAL

Proceed as directed under XVI, 3, 4, or 5, or calculate from the sp. gr. of the distillate from the Wichmann Lead Number, 9.

3

#### GLYCEROL—TENTATIVE

Proceed as directed under XVII, 26, 27, or 28, selecting the method according to the quantity of sugar present. Use such a quantity of the sample as contains 0.1–0.4 g of glycerol.

#### VANILLIN AND COUMARIN (GRAVIMETRIC)<sup>1</sup>—OFFICIAL

(This method is not applicable to concentrated vanillin and coumarin preparations in which the quantity of vanillin and coumarin present in 50 cc exceeds the quantity dissolved by 100 cc of H<sub>2</sub>O at 20°. With such preparations use a smaller quantity of the sample and dilute to 50 cc.)

4

#### PREPARATION OF SOLUTION

Measure 50 cc of the extract at 20° into a 250 cc beaker bearing marks showing volumes of 80 cc and 50 cc, dilute to 80 cc, and evaporate to 50 cc in a water bath kept at 70° or below. Dilute again with H<sub>2</sub>O to 80 cc and evaporate to 50 cc. Transfer to a 100 cc flask, rinsing the beaker with hot H<sub>2</sub>O, add 25 cc of 8% neutral Pb acetate soln, make up to the mark with H<sub>2</sub>O, shake, and allow to stand 18 hours (overnight) at 37–40°. Decant into a small, dry filter, reserving the filtrate (Soln A) for the determination of vanillin and coumarin (5), the Pb number (Winton, 8) and the residual color (19).

5

#### DETERMINATION

(a) *Vanillin*.—Transfer a 50 cc aliquot of the filtrate (Soln A) to a separatory funnel and extract with 4 successive 15 cc portions of ether (previously washed twice with an equal volume of H<sub>2</sub>O to remove alcohol). Wash the combined ether solns 4 or 5 times with NH<sub>4</sub>OH (1+11), using 10 cc the first time and 5 cc thereafter. Reserve the ether soln for the determination of coumarin. Slightly acidify the combined ammoniacal solns with HCl (1+2), cool, and extract in a separatory funnel with 4 portions of washed ether, using about 40 cc altogether. Evaporate the ethereal solns at room temp., dry over H<sub>2</sub>SO<sub>4</sub>, and weigh. (The vanillin residue often appears first in the form of oil-like droplets, which on standing crystallize into light colored masses.) If, after standing in a desiccator, the residue is considerably discolored or gummy, extract the vanillin from it by treating with at least 15 successive portions of boiling petroleum ether (b.p. 40° or below); combine the petroleum ether extracts, evaporate to dryness, and weigh.

The residue, if pure vanillin, should be white crystals melting at approximately 80°. Dissolve a small quantity of the residue in 2 drops of HCl and add a crystal of resorcin. Vanillin gives a pink coloration.

(b) *Coumarin*.—Evaporate at room temp. the original ether extract obtained

under (a), from which the vanillin has been removed by means of  $\text{NH}_4\text{OH}$  (1+1), dry over  $\text{H}_2\text{SO}_4$ , and weigh.

The residue, if pure coumarin, melts at approximately  $67^\circ$ . Dissolve a small quantity of the residue in not more than 0.5 cc of hot  $\text{H}_2\text{O}$  and add a few drops of 0.1 *N*  $\text{I}$  soln. Coumarin yields a brown precipitate which finally gathers in green flecks, leaving a clear brown colored soln. The reaction is especially marked if the reagent is applied with a glass rod to a few drops of the soln on a white plate or tile.

#### VANILLIN (COLORIMETRIC)<sup>2</sup>—OFFICIAL

6

##### REAGENT

*Phosphotungstic-phosphomolybdic acid.*—To 100 g of pure Na tungstate and 20 g of phosphomolybdic acid (free from nitrates and  $\text{NH}_4$  salts), add 100 g of sirupy  $\text{H}_3\text{PO}_4$  (containing 85%  $\text{H}_3\text{PO}_4$ ) and 700 cc of  $\text{H}_2\text{O}$ . Boil over a free flame for 1½–2 hours, cool, filter, if necessary, and make up with  $\text{H}_2\text{O}$  to 1 liter. An equivalent amount of pure molybdic acid may be substituted for the phosphomolybdic acid.

7

##### DETERMINATION

Transfer to a 100 cc volumetric flask a quantity of the sample that contains from 8–12 mg of vanillin (usually 5 cc). Add 75 cc of tap  $\text{H}_2\text{O}$  at room temp. and 4 cc of Pb soln (50 g each of basic and neutral Pb acetate per liter). Dilute to 100 cc with  $\text{H}_2\text{O}$  and mix. Filter thru a dry filter paper and pipet 5 cc of the clear filtrate into a 50 cc volumetric flask. Into another 50 cc volumetric flask pipet 5 cc of standard vanillin soln (1 cc = 0.1 mg of vanillin). To each of these flasks add from a pipet 5 cc of the reagent, allowing it to flow down the neck of the flask in such a way as to wash down the vanillin soln that may be on the sides of the flask. Mix the contents of the flasks by rotating and after 5 min. dilute the contents to 50 cc with saturated  $\text{Na}_2\text{CO}_3$  soln. Mix thoroly by inverting the flasks several times and allow to stand for at least 10 min. so that the precipitate that forms may separate completely. Filter the solns thru dry filter papers and compare the blue colors of the clear solns in a colorimeter. Report result as g of vanillin per 100 cc of extract.

#### LEAD NUMBER—OFFICIAL

8

##### I. Winton Method<sup>3</sup>

Determine Pb as sulfate or chromate, 10(a) or 10(b), in the filtrate from the Pb acetate precipitate (Soln A, 4) and in the filtrate from a blank determination, using  $\text{H}_2\text{O}$  and 5 drops of glacial acetic acid in place of the sample. Calculate the Pb number and report as "Lead Number—Winton."

9

##### II. Wichmann Method<sup>1</sup>

Place 175 cc of boiled  $\text{H}_2\text{O}$  in a round-bottomed flask of 1 liter capacity. Add by means of a pipet 25 cc of clear Pb acetate soln (8 g per 100 cc) and 50 cc of sample. Place the flask in a hole in an asbestos board that is large enough to prevent the heating of the upper portion of the flask. (When the contents of the flask are reduced to 50 cc of liquid, the level of the liquid should be even with the top of the board, or slightly above it.) Connect the flask to a condenser, and with a moderate flame distil 200 cc into a volumetric flask, reserving the distillate for the determination of alcohol. Transfer the residual soln to a 100 cc volumetric flask by means of  $\text{CO}_2$ -free  $\text{H}_2\text{O}$  and a bent glass rod provided with a rubber tip. When cool, dilute to 100 cc with  $\text{CO}_2$ -free  $\text{H}_2\text{O}$ , mix, and filter thru a dry filter (Soln A). Conduct a blank



determination, using 5 drops of glacial acetic acid in place of the sample and distilling 150 cc instead of 200 cc. Determine Pb as directed in 10(a) or 10(b) and calculate the Pb number and report as "Lead Number—Wichmann."

## 10

DETERMINATION OF LEAD<sup>1</sup>

(a) *As sulfate*.—Pipet 10 cc of Soln A (4 or 9) into a 250 cc beaker, add 25 cc of H<sub>2</sub>O, 2 cc of H<sub>2</sub>SO<sub>4</sub> (1+1) and 100 cc of 95% alcohol, stir, and allow to settle overnight. Filter on a Gooch crucible, wash with 95% alcohol, ignite at low redness, cool in a desiccator, and weigh. The difference between the weight of PbSO<sub>4</sub> obtained from the blank and that obtained from the sample  $\times 13.66$  = the Pb number of the extract.

(b) *As chromate*.—Pipet 10 cc of Soln A (4 or 9) into a 400 cc beaker and add 2 cc of glacial acetic acid, 25 cc of H<sub>2</sub>O, and 25 cc of approximately 0.1 N K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> soln. Heat the beaker and contents immediately with a moderate flame until the precipitate changes in color from yellow to orange. Filter on a Gooch crucible; wash thoroly with hot H<sub>2</sub>O and then with a few cc each of alcohol and ether. Dry at 100°, cool in a desiccator, and weigh. The difference between the weight of PbCrO<sub>4</sub> obtained from the blank and that obtained from the sample  $\times 12.82$  = the Pb number.

## 11

## TOTAL SOLIDS—OFFICIAL

Proceed as directed under XXXIV, 4, using 10 cc of the sample.

## 12

## ASH—OFFICIAL

Evaporate 10 cc of the extract and proceed as directed under XXVII, 8.

## 13

## ASH CONSTITUENTS

Proceed as directed under XII.

## 14

## SUCROSE—OFFICIAL

Proceed as directed under XXXIV, 22, 23, or 28.

## VANILLA RESINS

## 15

*Quantitative Method<sup>2</sup>—Tentative*

Pipet 50 cc of the extract into a small beaker, add 50 cc of H<sub>2</sub>O, and evaporate to 50 cc on the steam bath. Add 50 cc of H<sub>2</sub>O and again evaporate to 50 cc. Cool. If the mixture has an acid reaction, add 2 cc of HCl (1+1). If the mixture is not acid to litmus, add HCl (1+1), dropwise, until distinctly acid to litmus paper, then 1 cc in excess. Cover and let stand overnight. Filter, wash 6 or 7 times with approximately 0.05 N HCl, 9 cc of HCl (1+1) per liter of H<sub>2</sub>O. Dissolve the resin in warm 95% alcohol by pouring thru the filter. Evaporate the alcohol in a tared 50 cc beaker and dry to constant weight at 100°. Report results to 2 decimal places only. Reserve the resin for qualitative tests.

## 16

*Qualitative Test—Tentative*

Place a portion of the dried residue in a few cc of 5% KOH soln. Vanilla resins dissolve, giving a deep red soln. Acidify, and a precipitate is obtained.

Dissolve a portion of the dried residue in 95% alcohol. To a portion of the soln add a few drops of a 10% FeCl<sub>3</sub> soln; to another portion add HCl. Neither produces any marked change in color if the residue consists of vanilla resins. Most other resins in alcoholic soln give color reactions with FeCl<sub>3</sub> or HCl.

To a portion of the filtrate obtained in (15), add a few drops of basic Pb acetate soln, XXXIV, 18(a). Owing to the excessive quantity of organic acids, gums, and other extractive matter, the precipitate is so bulky as almost to solidify. The filtrate from this precipitate should be almost colorless.

Test another portion of the filtrate from the resin for tannin with a soln of gelatin. Tannin is present in varying but small quantities, but should not be present in excessive quantities.

17

METHYL ALCOHOL—OFFICIAL

Proceed as directed under XVI, 19, 20, or 23, using the distillate from the determination of alcohol under 2.

18

COLOR VALUE—TENTATIVE

Pipet 2 cc of the extract into a 50 cc volumetric flask and dilute to the mark with a mixture of equal parts of 95% alcohol and H<sub>2</sub>O. Determine the color value of this diluted extract in terms of red and yellow by means of a Lovibond tintometer, using a 1-inch cell. To obtain the color value of the original extract, multiply the figures for each color by 25.

19 RESIDUAL COLOR AFTER PRECIPITATION WITH LEAD ACETATE—TENTATIVE

Determine the color value, in terms of red and yellow, of the filtrate from the Pb acetate precipitate obtained under 4, using a 1-inch Lovibond cell. Multiply the reading by 2 to reduce the results to the basis of the original extract. If the actual reading of the soln is greater than 5 red and 15 yellow, as may be the case if the extract is highly colored with caramel, use a half or quarter inch cell, and multiply the readings, respectively, by 4 or 8. To obtain the percentages of the two colors remaining in the Pb acetate filtrate, divide the figures for red and yellow, respectively, by the corresponding figures of the original extract obtained under 18 and multiply the quotients by 100. Calculate also the ratio of red to yellow in both extract and Pb acetate filtrate.

20

COLORS INSOLUBLE IN AMYL ALCOHOL—TENTATIVE

Proceed as directed under XVII, 79, using 25 cc of the extract and shaking with 25 cc of the Marsh reagent instead of 20 cc.

21

COLORING MATTERS OTHER THAN CARAMEL—TENTATIVE

Proceed as directed under XXI.

LEMON AND ORANGE EXTRACTS

22

SPECIFIC GRAVITY—OFFICIAL

Determine at 20/20° by means of a pycnometer, as directed under XIV, 3.

ALCOHOL

23

Method I.—Official

Pipet 50 cc of the extract into a 200 cc volumetric flask, noting the temp.; dilute with H<sub>2</sub>O to approximately 200 cc; and allow the mixture to stand until the oil separates in a clear layer at the top, or centrifuge and add H<sub>2</sub>O to bring the lower meniscus of the oil to the mark. Pour the mixture into a dry Erlenmeyer flask containing 5 g of light MgCO<sub>3</sub>, stopper, shake well, and filter quickly thru a large, dry, folded filter. Introduce a 100 cc aliquot of the filtrate, measured at the same temp.,

into a 300–500 cc distillation flask, and add 50 cc of  $H_2O$ . Attach the flask to a condenser and distil almost 100 cc. Add  $H_2O$  to complete the volume of the distillate to 100 cc at the same temp., mix well, and determine the sp. gr. at a convenient temp. Ascertain the corresponding percentage of alcohol by volume from **XLII**, Table 19, and multiply the result thus obtained by 4 to obtain the percentage of alcohol by volume in the original sample.

## 24

*Method II.—Official*

(Applicable to extracts consisting only of oil, alcohol, and water.)

Let  $S$  represent the sp. gr. of the extract at  $20/20^\circ$ , as determined under **22**;  $O$ , the sp. gr. of the oil; and  $p$ , the percentage of oil found. Then  $100 - p$  = the percentage of the water-alcohol soln, the sp. gr. of which, represented by  $P$ , is calculated as follows:

$$S = \frac{Op + P(100 - p)}{100}, \text{ whence } P = \frac{100S - Op}{100 - p}.$$

The value of  $E$ , the alcohol equivalent of  $P$ , is obtained from **XLII**, Table 19. It gives the percentage of alcohol in the alcohol-water soln. To find the percentage of alcohol in the extract, apply the following formula:

$$\text{Percentage by volume of alcohol in the extract} = E \left( 1 - \frac{p}{100} \right).$$

The value of  $O$  for lemon oil may be taken as 0.86 and for orange oil as 0.85.

## 25

## GLYCEROL—TENTATIVE

Proceed as directed under **XVII**, **26**, **27** or **28**, selecting the method according to the quantity of sugar present. Use a quantity of the sample that contains 0.1–0.4 g of glycerol.

## OILS OF LEMON AND ORANGE IN EXTRACTS

## 26

*I. By Polarization—Official*

Without diluting, polarize the extract at  $20^\circ$  in a 200 mm tube. Divide the reading in degrees Ventzke by 3.2 in the case of lemon extract and by 5.2 in the case of orange extract; in the absence of other optically active substances, the result will be the percentage of oil by volume. If cane sugar is present, determine as directed under **34** and correct the reading accordingly. To obtain the percentage of oil by weight from the percentage by volume, multiply the volume percentage by 0.86 in the case of lemon extracts, and by 0.85 in the case of orange extracts, and divide the results by the sp. gr. of the original extract.

## 27

*II. By Precipitation—Official*

Pipet 20 cc of the extract into a Babcock milk bottle. Add 1 cc of  $HCl$  (1+1), then 25–28 cc of  $H_2O$  previously warmed to  $60^\circ$ . Mix, and let stand in  $H_2O$  at  $60^\circ$  for 5 min. Centrifuge for 5 min., fill the bottle with warm  $H_2O$  to bring the oil into the graduated neck of the flask, again centrifuge for 2 min., and place the flask in  $H_2O$  at  $60^\circ$  for a few minutes. Note the percentage of oil by volume. If oil is present in amounts over 2%, add 0.4% to the percentage of oil noted to correct for the solubility of the oil. If less than 2% and more than 1% is present, add 0.3% for this correction. To obtain the percentage of oil by weight from the percentage by volume, multiply the volume percentage by 0.86 in the case of lemon extracts, and by 0.85 in the case of orange extracts, and divide the result by the sp. gr. of the original extract.

TOTAL ALDEHYDES<sup>a</sup>—OFFICIAL

28

## REAGENTS

(a) *Aldehyde-free alcohol*.—Allow 95% alcohol, containing 5 g of metaphenylenediamine hydrochloride per liter, to stand for at least 24 hours with frequent shaking. (Nothing is gained by previous treatment with KOH.) Boil under a reflux condenser for at least 8 hours, longer if necessary; allow to stand overnight, and distil, rejecting the first 10 and the last 5 cc which come over. Store in a dark, cool place in well-filled bottles; 25 cc of this alcohol, on standing 20 min. at 14–16° with 20 cc of the sulfite-fuchsin soln, should develop only a faint pink coloration. If a stronger color is developed, repeat the treatment with metaphenylenediamine hydrochloride as above.

(b) *Sulfite-fuchsin soln*.—Dissolve 0.5 g of fuchsin in 250 cc of H<sub>2</sub>O, add an aqueous soln of SO<sub>2</sub> containing 16 g of the gas, allow to stand until colorless or nearly so, and make up to 1 liter with H<sub>2</sub>O. Let stand 12 hours before using and keep in a refrigerator. This soln is liable to deteriorate and should be reasonably fresh when used.

(c) *Standard citral soln*.—Weigh 0.5 g of citral into a 50 cc volumetric flask, make up to mark with the aldehyde-free alcohol at room temp., stopper the flask, and mix by shaking. Dilute 10 cc of this soln with the aldehyde-free alcohol to 100 cc in a volumetric flask, stopper the flask, and mix by shaking. 1 cc of the dilute soln = 1 mg of citral.

29

## DETERMINATION

Weigh approximately 25 g of the extract in a stoppered weighing flask, transfer to a 50 cc volumetric flask, and dilute to the mark at room temp. with aldehyde-free alcohol. Measure, at room temp., 2 cc (or other suitable quantity) of this soln into a comparison tube. Add 25 cc of the aldehyde-free alcohol (previously cooled to 14–16°), then 20 cc of the sulfite-fuchsin soln (also cooled), and finally make up to the 50 cc mark with the aldehyde-free alcohol. Mix thoroly, stopper, and keep at 14–16° for 15 min. Prepare a standard for comparison at the same time and in the same manner, using 2 cc of the standard citral soln, and compare the colors developed. Calculate the amount of citral present and repeat the determination, using a quantity sufficient to give the sample approximately the strength of the standard. From this result calculate the quantity of citral in the sample. If the comparisons are made in Nessler tubes, standards containing 1, 1.5, 2, 2.5, 3, 3.5, and 4 mg of citral may be prepared and the trial comparison made against these, the final comparison being made with standards lying between 1.5 and 2.5 mg with 0.25 mg increments.

It is absolutely essential to keep the reagents and comparison tubes at the required temp., 14–16°. If the comparisons are made in a bath (this being possible only when the bath is of glass), the standards should be discarded within 25 min. after adding the sulfite-fuchsin soln. Give samples and standards identical treatment.

CITRAL<sup>a</sup>—OFFICIAL

(Lemon and orange extracts.)

30

## REAGENT

*Metaphenylenediamine hydrochloride-oxalic acid soln*.—Dissolve 1 g of metaphenylenediamine hydrochloride in about 45 cc of 85% alcohol, and 1 g of crystallized oxalic acid in a similar quantity of alcohol of the same strength, and pour the two solns into a 100 cc volumetric flask. Add 2 or 3 g of fullers' earth, dilute to the mark with 85% alcohol, mix, and filter thru a double folded filter.

**31 DETERMINATION**

Weigh 25 g of the extract into a 50 cc volumetric flask, dilute to the mark with alcohol (95% by volume for extracts made with the oils; 50-95% by volume for terpeneless extracts) and mix. Pipet 2 cc or other suitable quantity of this soln into a colorimeter tube, add 10 cc of the reagent, dilute to suitable volume, and compare the resulting color with the colors of a set of standards containing known quantities of standard citral soln, 28(c).

**32 TOTAL SOLIDS—OFFICIAL**

Proceed as directed under XVII, 62, using 10 cc of the sample measured at 20°.

**33 ASH—OFFICIAL**

Ignite the residue from 10 cc of the extract as directed under XXVII, 8.

**34 SUCROSE—OFFICIAL**

Neutralize the normal weight of the extract, evaporate to dryness, wash several times with ether, dissolve in H<sub>2</sub>O, and proceed as directed under XXXIV, 22, 23 or 28.

**35 METHYL ALCOHOL—OFFICIAL**

Proceed as directed under XVI, 19, 20, or 23, using the distillate from the determination of alcohol under 23.

**36 COLORING MATTERS—TENTATIVE**

Proceed as directed under XXI.

**37 LEMON AND ORANGE PEEL COLOR—TENTATIVE**

Place a few cc of the extract in each of 2 test tubes; to one, add slowly 3-4 volumes of HCl and to the other, several drops of NH<sub>4</sub>OH. If the color is due to lemon or orange peel only, it is materially deepened by each treatment.

**LEMON AND ORANGE OILS****38 SPECIFIC GRAVITY—OFFICIAL**

Determine the sp. gr. at 20°/20° by means of a pycnometer, as directed under XIV, 3.

**39 INDEX OF REFRACTION OFFICIAL**

Use any standard instrument, making the reading at 20° (XXXI, 8).

**40 OPTICAL ROTATION—OFFICIAL**

Use 20° with any standard instrument, a 50 mm tube, and Na light. The results should be stated in angular degrees on a 100 mm basis. If instruments having the sugar scale are used, the reading for orange oils is above the range of the scale, but readings may be obtained by the use of standard levorotatory quartz plates, or by the use of a 25 mm tube. The true rotation cannot be obtained by diluting the oil with alcohol and correcting the rotation in proportion to the dilution.

**41 TOTAL ALDEHYDES\*—OFFICIAL**

Weigh a small quantity of the sample into a small stoppered flask and dilute with aldehyde-free alcohol in the proportion of 2 g of lemon oil or 4 g of orange oil to 10 cc of soln. Determine the total aldehydes as directed under 29, expressing the result as citral.

*Kleber Method*<sup>11</sup>—Official

42

## REAGENT

*Phenylhydrazine soln.*—Prepare a 10% soln in absolute alcohol. A sufficiently pure reagent can be obtained by distilling the commercial product in vacuo, rejecting the first portions coming over that contain  $\text{NH}_3$ .

43

## DETERMINATION

Weigh accurately about 15 g of the sample into a small, glass-stoppered flask, and add 10 cc of the phenylhydrazine soln. Allow to stand 30 min. at room temp. and titrate with 0.5 N HCl, using either methyl or ethyl orange indicator. Titrate similarly 10 cc of the phenylhydrazine soln. The difference in the number of cc of 0.5 N acid used in these 2 titrations  $\times$  the factor 0.076 = the weight of citral in the sample. If difficulty is experienced in detecting the end point of the reaction, titrate until the soln is distinctly acid; transfer to a separatory funnel and draw off the alcoholic portion. Wash the oil with  $\text{H}_2\text{O}$ , adding the washings to the alcoholic soln, titrate back with 0.5 N alkali, and make the necessary corrections.

44

*Hiltner Method*<sup>11</sup>—Official

Weigh accurately about 2 g of lemon oil or 8 g of orange oil into a 100 cc volumetric flask, dilute to the mark with 95% alcohol, and proceed as directed under 31, using 2 cc of the dilute soln for the comparison.

45

PHYSICAL CONSTANTS OF THE 10 PER CENT DISTILLATE<sup>12</sup>—OFFICIAL

Place 50 cc of the sample in a 3-bulb Ladenburg flask having the main bulb 6 cm in diameter and of 120 cc capacity and the condensing bulbs of the following dimensions: 3.5 cm, 3 cm, and 2.5 cm. The distance from the bottom of the flask to the opening of the side arm should be 20 cm. Distil the oil at the rate of 2 cc per min. until 5 cc has been distilled. Determine the refractive index and rotation of this distillate as directed under 39 and 40.

46

PINENE<sup>13</sup>—OFFICIAL

Mix the 10% distillate, 45, with 5 cc of glacial acetic acid, cool the mixture thoroly in a freezing bath, and add 10 cc of ethyl nitrite. Then add slowly, with constant stirring, 2 cc of  $\text{HCl}$  (2+1). Keep the mixture in the freezing bath 15 min. Collect the crystals formed on a filter, using suction, and wash with 95% alcohol. Return the combined filtrate and washings to the freezing bath for 15 min. Collect the additional crystals formed on the original filter. Wash the combined crops of crystals thoroly with alcohol. Dry at room temp. and dissolve in a minimum quantity of  $\text{CHCl}_3$ . Add methyl alcohol to the  $\text{CHCl}_3$  soln, a little at a time, until the nitroso-chlorides crystallize out. Mount the separated and dried crystals in olive oil and examine under the microscope. Pinene nitroso-chloride crystals have irregular pyramidal ends, while limonene nitroso-chloride crystallizes in needles.

## ALMOND EXTRACT

47

## ALCOHOL—TENTATIVE

As almond extract usually contains only about 1% of almond oil, in most cases the alcohol can be calculated from the sp. gr. of the extract. If the extract is high in solids, proceed as follows: Add 25 cc of the extract, measured at room temp., to 75 cc of saturated NaCl soln in a separatory funnel, and extract twice with 50 cc

portions of petroleum ether (b.p. 40–60°). Collect the petroleum ether extract in a second separatory funnel, and wash twice with 2 portions (25 cc) of saturated brine. Combine the original salt soln with the washings, add a little powdered pumice, and distil into a 100 cc volumetric flask. When almost 100 cc has been distilled, make up to the mark with H<sub>2</sub>O at room temp. and determine alcohol from the sp. gr. as directed under XIV, 3, using Table 19, XLII.

## 48

## BENZALDEHYDE—TENTATIVE

Measure out 2 portions of 10 cc each of the extract into 300 cc Erlenmeyer flasks and add 10 cc of phenylhydrazine soln (add 3 cc of glacial acetic acid to 40 cc of H<sub>2</sub>O and mix with 2 cc of phenylhydrazine) to one flask and 15 cc to the other. Allow the mixtures to stand overnight in a dark place. Then add 200 cc of H<sub>2</sub>O, and filter thru a weighed Gooch crucible provided with a thin layer of asbestos. Wash the precipitate first with cold H<sub>2</sub>O and finally with 10 cc of 10% alcohol. Dry at 70° for 3 hours at a pressure not to exceed 100 mm of Hg or to constant weight over H<sub>2</sub>SO<sub>4</sub>. Weight of the precipitate  $\times$  the factor 5.408 = the weight of benzaldehyde in 100 cc of the sample. If duplicate determinations do not agree, repeat the operation, using a larger quantity of the phenylhydrazine soln.

## 49

## BENZOIC ACID—TENTATIVE

Measure 10 cc of the extract into a 100 cc flask and add 10 cc of a 10% NaOH soln and 20 cc of 3% H<sub>2</sub>O<sub>2</sub> soln; cover with a watch-glass and place in a water oven. Oxidation of the aldehyde to benzoic acid begins almost immediately and should continue 5–10 min. after all odor of benzaldehyde has disappeared (usually 20–30 min.). Remove the flask from the water oven; transfer the contents to a separatory funnel, rinsing off the watch-glass; add 10 cc of H<sub>2</sub>SO<sub>4</sub> (1+5), and cool the contents of the funnel to room temp. under the water tap. Extract the benzoic acid with 4 portions of 25, 25, 20, and 20 cc of ether, respectively, and wash the combined extracts with 2 portions of 5–10 cc of H<sub>2</sub>O, or until all H<sub>2</sub>SO<sub>4</sub> is removed. Filter into a weighed dish, evaporate at room temp., dry overnight in a desiccator, and weigh the benzoic acid. Multiply the result by 10.

Multiply the grams per 100 cc of benzaldehyde obtained under 48 by 1.151 to obtain the equivalent of benzoic acid and subtract this product from the grams per 100 cc of total benzoic acid obtained above. The difference is the grams of benzoic acid per 100 cc of the extract.

## HYDROCYANIC ACID

## 50

*Qualitative Test—Tentative*

Add several drops of a freshly prepared 3% FeSO<sub>4</sub> soln and a single drop of 1% FeCl<sub>3</sub> soln to several cc of the extract. Mix thoroly and add 10% NaOH soln, dropwise, until no further precipitate forms and then H<sub>2</sub>SO<sub>4</sub> (1+9) to dissolve the precipitate. In the presence of even small quantities of HCN, a Prussian blue coloration or suspension will develop.

## 51

*Quantitative Method—Tentative*

(In the absence of chlorides.)

Measure 25 cc of the extract into a small flask and add 5 cc of freshly precipitated Mg(OH)<sub>2</sub>, Cl-free. Titrate with 0.1 N AgNO<sub>3</sub> soln, using K<sub>2</sub>CrO<sub>4</sub> as an indicator. 1 cc of 0.1 N AgNO<sub>3</sub> = 0.0027 g of HCN.

## NITROBENZOL

52

*Qualitative Test—Tentative*

Boil a few cc of the extract with some Zn dust and acetic acid and filter. Add to the filtrate a drop of  $\text{CHCl}_3$ , make strongly alkaline with 10% NaOH soln, and heat. The presence of nitrobenzol in the original extract is indicated by the development of the characteristic odor of phenylisocyanide.

## CASSIA, CINNAMON, AND CLOVE EXTRACTS

53

## ALCOHOL—TENTATIVE

Proceed as directed under 47.

OIL<sup>14</sup>

54

*Method I.—Tentative*

Transfer 10 cc of the extract to a separatory funnel, add 30 cc of  $\text{H}_2\text{O}$ , acidify with 1 cc of HCl (1+1), and extract 3 times with ether, using not less than 100 cc altogether. Wash the combined ether solns twice with  $\text{H}_2\text{O}$ , and in the case of cinnamon extract dry by shaking with a small quantity of granulated  $\text{CaCl}_2$ . Transfer to a weighed wide-mouthed weighing bottle and evaporate the ether as rapidly as possible on a boiling water bath, rotating the liquid upon the sides of the bottle in order to rid the residual oil of traces of ether. Weigh the residue and divide the weight by the sp. gr. of the oil in order to obtain the percentage of oil by volume. In the case of clove oil, allow the weighing bottle to remain in the balance case until the usual film of moisture has evaporated. The time of weighing, however, should not be delayed over 3 min. Determine the refractive index of the residual oils at 20°. Dissolve a drop of the oil in several drops of alcohol and add a drop of 10%  $\text{FeCl}_3$  soln.

*Specific gravity, refractive index at 20°, and color reaction with  $\text{FeCl}_3$  soln*

OIL	SPECIFIC GRAVITY	REFRACTIVE INDEX AT 20°	COLOR REACTION WITH $\text{FeCl}_3$ SOLN
Cassia .....	1.05	1.585–1.600	Brown
Cinnamon .....	1.03	1.590–1.599	Green
Cloves .....	1.055	1.560–1.565	Deep blue

55

*Method II.<sup>15</sup>—Tentative*

(Applicable to extracts of cinnamon and clove.)

Pipet 10 cc of extract into a standard Babcock milk bottle. Remove nearly all alcohol by blowing air into the bottle thru a small glass tube for 30 min., or longer if necessary. Add from a 10 cc buret 1 cc of solvent (equal parts of U.S.P. mineral oil and  $\text{H}_2\text{O}$ -free kerosene), shake well, and fill with a saturated soln of  $\text{MgSO}_4$ . Centrifuge for 10 min. and read the volume of oil from the extreme bottom to the extreme top of the column. To obtain the percentage of oil subtract 5 divisions and multiply the remainder by 2.

## GINGER EXTRACT

56

## ALCOHOL TENTATIVE

Proceed as directed under XVI, 3, 4 or 5.



## 57

## SOLIDS—TENTATIVE

Evaporate 10 cc of the extract nearly to dryness on the steam bath, dry for 2 hours in a water oven at the temp. of boiling  $H_2O$ , and weigh.

## 58

## GINGER (QUALITATIVE TEST)—TENTATIVE

Dilute 10 cc of the extract to 30 cc, evaporate to 20 cc, decant into a separatory funnel, and extract with an equal volume of ether. Allow the ether to evaporate spontaneously in a porcelain dish, and to the residue add 5 cc of 75%  $H_2SO_4$  and about 5 mg of vanillin. Allow to stand 15 min. and add an equal volume of  $H_2O$ . In the presence of ginger extract an azure blue color develops.

## 59

## CAPSICUM (QUALITATIVE TEST)—TENTATIVE

To 10 cc of the extract add cautiously  $NaOH$  soln (1+9) until the soln reacts very slightly alkaline with litmus paper. Evaporate at about  $70^\circ$  to approximately  $\frac{1}{2}$  the original volume and render slightly acid with  $H_2SO_4$  (1+9), testing with litmus paper. Transfer to a separatory funnel, rinsing the dish with  $H_2O$ , and extract with an equal volume of ether, avoiding the formation of an emulsion by shaking the funnel gently 1-2 min. Draw off the lower layer and wash the ether extract once with about 10 cc of  $H_2O$ . Transfer the washed ether extract to a small evaporating dish, render decidedly alkaline with 0.5  $N$  alcoholic  $KOH$ , and evaporate at about  $70^\circ$  until the residue is pasty. Then add about 20 cc more of the 0.5  $N$  alcoholic  $KOH$  and allow to stand on the steam bath until the gingerol is completely saponified (about 30 min.). Dissolve the residue in a little  $H_2O$  and transfer with  $H_2O$  to a small separatory funnel. The volume should not exceed 50 cc. Extract the alkaline soln with an equal volume of ether. Wash the ether extract repeatedly with small quantities of  $H_2O$  until no longer alkaline to litmus. Transfer the washed extract to a small evaporating dish and allow the ether to evaporate spontaneously. Finally test the residue for capsicum by moistening the tip of the finger, rubbing it on the bottom and sides of the dish, and then applying the finger to the end of the tongue. A hot, stinging, or prickly sensation, which persists for several minutes, indicates capsicum or other foreign pungent substances.

## PEPPERMINT, SPEARMINT, AND WINTERGREEN EXTRACTS

## 60

## ALCOHOL—TENTATIVE

Proceed as directed under 47.

## 61

OIL<sup>14</sup>—TENTATIVE

Pipet 10 cc of the extract into a Babcock milk bottle, add 1 cc of  $CS_2$ , mix thoroly, and add 25 cc of cold  $H_2O$  and 1 cc of  $HCl$ . Close the mouth of the bottle and shake vigorously; centrifuge for 6 min., and remove all but 3-4 cc of the supernatant liquid, which should be practically clear, by aspirating thru a glass tube of small bore. Connect the stem of the bottle with a filter pump, immerse the bottle in  $H_2O$  kept at approximately  $70^\circ$  for 3 min., remove from the bath every 15 seconds, and shake vigorously. Continue in the same manner for 45 seconds, using a boiling water bath. Remove from the bath and shake while cooling. Disconnect from the suction and fill the bottle to the neck with saturated salt soln at room temp., centrifuge for 2 min., and read the volume of the separated oil from the top of the meniscus. Multiply the reading by 2 to obtain the percentage of oil by volume. In the case of wintergreen, use as a floating medium a mixture of 1 volume of  $H_2SO_4$  and 3 of saturated  $Na_2SO_4$  soln.

62 METHYL SALICYLATE IN WINTERGREEN EXTRACT<sup>14</sup>—TENTATIVE

Mix 10 cc of the extract with 10 cc of 10% KOH soln. Heat on a steam bath until the volume is reduced about one-half. Add a distinct excess of dilute HCl (1+1), cool, and extract with 3 portions of ether, 40, 30, and 20 cc, respectively. Filter the extract thru a dry filter into a weighed dish, wash the paper with 10 cc of ether, and allow the filtrate and washings to evaporate spontaneously. Dry in a desiccator containing  $\text{H}_2\text{SO}_4$  and weigh. Weight of salicylic acid so found  $\times 9.33$  = the percentage by volume of methyl salicylate in the sample.

## ANISE AND NUTMEG EXTRACTS

63

OIL<sup>14</sup>—TENTATIVE

To 10 cc of the extract in a Babcock milk bottle, add 1 cc of HCl (1+1), then sufficient half-saturated salt soln, previously heated to 60°, to fill the flask nearly to the neck. Cork and let stand in  $\text{H}_2\text{O}$  at 60° for about 15 min., rotate occasionally, and centrifuge for 10 min. at about 800 r.p.m. Add brine till the oil rises into the neck of the bottle and again centrifuge for 10 min. If the separation is not satisfactory or the liquid is not clear, cool to about 10° and centrifuge for an additional 10 min. The reading  $\times 2$  = the percentage of oil by volume.

ESSENTIAL OIL IN EXTRACTS AND TOILET PREPARATIONS<sup>15</sup>

- 64 Applicable to extracts of allspice, anise, caraway, lemon, nutmeg, orange, peppermint, pimiento, rosemary, thyme, wintergreen and methyl salicylate.

Pipet 10 cc of sample (5 cc when the oil content exceeds 5% by volume) into a standard Babcock milk bottle, add 0.50 cc of solvent (equal parts of U.S.P. mineral oil and  $\text{H}_2\text{O}$ -free kerosene) and 1 cc of HCl (1+1), and fill to the shoulder with a saturated NaCl soln. Shake the bottle for 3 min., then add the salt soln to bring the column of oil within the graduations on the neck. Centrifuge for 10 min. at high speed and read the volume of oil from the extreme bottom to the extreme top of the column. (Read from extreme bottom to the bottom of the meniscus at the top of the column for allspice, peppermint, and pimiento extracts.) To obtain the percentage of oil subtract 2.5 divisions and multiply the remainder by 2. (Multiply by 4 if a 5 cc sample is used.)

## OILS OF LEMON, ORANGE, AND LIMES IN VEGETABLE AND MINERAL OILS

1. By Steam Distillation<sup>18</sup>—Official

65

## APPARATUS

(a) *Steam generator filled with  $\text{H}_2\text{O}$ .*—An oil can holding 1 gallon will serve the purpose.

(b) *Distillation flask.*—A Kjeldahl flask of about 750 cc capacity, with shortened neck, about 10 inches in height over all.

(c) *Spray tube.*—A glass tube with a small perforated bulb at the end passes thru a rubber stopper and reaches to the bottom of the distillation flask.

(d) *Bent glass tube.*—About 8 mm in diameter. Connects distillation flask to up-right condenser. The shape of this tube allows the vapor condensing in the tube to return to the distillation flask.

(e) *Liebig condenser.*—With 20-inch water jacket.

(f) *Wilson receiving flask.*—Shaped like a Babcock test bottle with a graduated neck but of much larger capacity and with a vertical glass outlet tube sealed on near the bottom. The upper end of the outlet tube is turned down. The capacity of the flask is about 250 cc. The neck may consist of a portion of a buret graduated from

0-25 cc with top flared out. The outlet tube is about 3 mm in diameter, and the end is at such a height that when the flask is filled with  $H_2O$  the meniscus in the neck will be between the 0 and 1 cc marks.

66

## DETERMINATION

Measure 100 cc of the sample in a graduated cylinder and transfer to the distillation flask. Immerse the flask in a water bath and connect with the condenser by means of the bent glass tube. Fill the receiving flask with  $H_2O$  and place under the condenser in such a way that the end of the condenser will be about 0.5 inch above the level of the  $H_2O$  in the receiving flask. Place a 200 cc graduated cylinder under the end of the outlet tube to catch the displaced liquid. Heat the water bath to boiling and pass steam thru the sample until 200 cc of liquid has been collected in the graduated cylinder.

Disconnect the apparatus, allow the receiving flask to stand for 15 min., or until separation of oil is complete, and read the volume of oil obtained. Calculate the percentage (by volume) of essential oil in the sample by dividing the reading by 0.90 for lemon oil in corn and cottonseed oils, 0.95 for orange oil in corn and cottonseed oils, and by 0.78 for distilled or expressed oil of limes in corn and cottonseed oils. Where the menstruum is mineral oil, subtract 0.3 cc from the reading before dividing by the factors 0.90, 0.95, and 0.78 for lemon oil, orange oil, and oil of limes, respectively.

67

*II. By Polarization*<sup>19</sup>—*Tentative*

Polarize the sample at 20° in a 200 mm tube, making 5 readings. From the average of these readings in degrees Ventzke subtract, for corn oil +0.6°, for cottonseed oil -0.3°, for peanut oil +0.2°, and for mineral oil +5.5°, as a correction for the rotatory effect of the menstruum. To obtain the percentage by volume of the essential oil in the mixture, divide the corrected polariscopic reading so obtained by the factor 3.4 for lemon oil in corn oil, 3.7 for lemon oil in cottonseed oil, 3.6 for lemon oil in peanut oil, 3.5 for lemon oil in mineral oil, 5.4 for orange oil in corn oil, 5.7 for orange oil in cottonseed oil, 5.6 for orange oil in mineral oil, 2.0 for oil of limes in corn oil, 2.3 for oil of limes in cottonseed oil, and 2.2 for oil of limes in mineral oil.

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- <sup>8</sup> J. Am. Chem. Soc., **28**, 1472 (1906); J. Assoc. Official Agr. Chem., **14**, 68 (1931).
- <sup>9</sup> J. Assoc. Official Agr. Chem., **12**, 48 (1929).
- <sup>10</sup> J. Am. Chem. Soc., **28**, 1472 (1906).
- <sup>11</sup> U. S. Dept. Agr. Bur. Chem. Bull., **137**, p. 72.
- <sup>12</sup> Schimmel and Co., Semi-annual Rpt., Oct. 1898, p. 41.
- <sup>13</sup> U. S. Dept. Agr. Bur. Chem. Circ. **46**, p. 9.
- <sup>14</sup> J. Ind. Eng. Chem., **1**, 84 (1909).
- <sup>15</sup> J. Assoc. Official Agr. Chem., **15**, 539 (1932); **16**, 75 (1933).
- <sup>16</sup> J. Ind. Eng. Chem., **3**, 252 (1911).
- <sup>17</sup> J. Assoc. Official Agr. Chem., **15**, 539 (1932); **16**, 75 (1933); **19**, 75 (1936).
- <sup>18</sup> *Ibid.*, **9**, 450 (1926); **11**, 45, 503 (1928).
- <sup>19</sup> *Ibid.*, **9**, 453 (1926); **10**, 495 (1927).

## XXVI. FRUITS AND FRUIT PRODUCTS

1

### SAMPLING—TENTATIVE

*Boxed fruit.*—Remove cover, bottom, or one side of box, as is most convenient. Remove a block comprising  $\frac{1}{2}$  of the contents of the box taken from one corner as follows: With a sharp knife make a vertical cut midway between the *ends* of the box to the center of the top surface, this cut to extend half way to the bottom. Make another vertical cut midway between the *sides* of the box, extending half way to the bottom, and continue it until it meets the first cut. Remove all fruit included in the angle formed by the two cuts. Working rapidly, break up all lumps, thoroly mix, and take sufficient sample to fill a quart Mason jar, replacing the remainder in the box. Seal the jar and send to the laboratory. Sample a sufficient number of boxes taken from different parts of the pile to constitute at least the square root of the lot.

2

### PREPARATION OF SAMPLE—OFFICIAL

Transfer all samples received in open packages (i.e., not in sterile condition) without delay to glass-stoppered containers and keep in a cool place. Make the determinations of alcohol, total and volatile acids, solids, and sugars, particularly in the case of fruit juices and fresh fruits, at once, as fermentation is liable to begin very soon. (Portions for the determination of sucrose and reducing sugars may be weighed and kept for several days without fermenting if the slight excess of neutral Pb acetate soln required in the determination is added.) The various products are prepared for analysis as follows:

(a) *Juices.*—Mix thoroly by shaking to insure uniformity in sampling and filter thru muslin previously washed and dried. Prepare fresh juices by pressing the well-pulped fruit in a jelly bag and filtering thru muslin previously washed and dried. Express the juice of citrus fruit by means of one of the common devices for squeezing oranges or lemons, and strain the expressed juice thru muslin previously washed and dried.

(b) *Jellies and sirups.* Mix thoroly to insure uniformity in sampling.

(b<sub>1</sub>) *Preparation of soln.*—Weigh 300 g of the thoroly mixed sample into a 2 liter flask and dissolve in H<sub>2</sub>O, heating on a steam bath, if necessary. Apply as little heat as possible to minimize inversion of the sucrose. Cool, dilute to the mark, mix thoroly by shaking, and use aliquots for the various determinations. If insoluble material is present, mix thoroly and filter before taking the aliquots.

(c) *Fresh fruits, dried fruits, canned fruits, preserves, jams, and marmalades.*—Pulp by grinding in a large mortar or by passing thru a food chopper and mix thoroly, completing the operation as quickly as possible to avoid loss of moisture. In the case of dried fruits, pass the sample thru the food chopper three times, mixing thoroly after each grinding. In the case of stone fruits, remove the pits, and determine their proportion in a weighed sample. In the case of canned fruits, an examination of the sirup in which the fruits are preserved is often sufficient. Separate the liquor by draining (cf. XXXV, 2) and treat as directed under (a).

(c<sub>1</sub>) *Preparation of soln.*—Weigh 300 g of the well-pulped and mixed sample into a 1.5-2-liter beaker; add about 800 cc of H<sub>2</sub>O; and boil 1 hour, replacing at intervals the H<sub>2</sub>O lost by evaporation. Transfer to a 2 liter volumetric flask, cool, dilute to volume, and filter. With unsweetened fruit it is desirable, tho not actually necessary, that sugar be added before boiling; therefore weigh 150 g of fruit, add 150 g of sugar and 800 cc of H<sub>2</sub>O and proceed as directed above.

3

## ALCOHOL—OFFICIAL

Determine alcohol in 50 g of the original material as directed under XIV, 5.

4

MOISTURE<sup>2</sup>—OFFICIAL

*Dried fruits.*—Spread 5–10 g of the prepared sample, 2(c), as evenly as possible over the bottom of a metal dish approximately 8.5 cm in diameter and provided with a tightly fitted cover; weigh; and dry at 70° for 6 hours under a pressure not to exceed 100 mm of Hg. During the drying admit to the oven a slow current of air (about 2 bubbles per second) dried by passing thru H<sub>2</sub>SO<sub>4</sub>. (The metal dish must be placed in direct contact with the metal shelf of the oven.) Replace the cover, cool in a desiccator, and weigh. Disregard any temporary drop of oven temp. that may occur during the early part of the drying period owing to rapid evaporation of H<sub>2</sub>O. With raisins and fruit similarly rich in sugar, use about 5 g of sample and dry and weigh with the dish about 2 g of finely divided asbestos. Moisten with hot H<sub>2</sub>O, mix the sample and asbestos thoroly, evaporate on a steam bath barely to dryness, and complete drying as directed above.

## TOTAL SOLIDS—OFFICIAL

5

*Method I. Insoluble Matter Present*

*Fresh and canned fruits, jams, marmalades and preserves.*—Weigh accurately 20 g of pulped fresh fruit, or a quantity of fruit products that will give not more than 3–4 g of dry material, into a large flat-bottomed dish. If necessary to secure a thin layer of the material, add a few cc of H<sub>2</sub>O and mix thoroly. Dry at 70° under a pressure of not to exceed 100 mm of Hg until consecutive weighings made at intervals of 2 hours do not vary more than 3 mg.

6

*Method II. No Insoluble Matter Present*

*Fruit juices, jellies and sirups.*—Proceed as directed under XXXIV, 3, 5, 6 or 7, using the sample prepared as directed under 2(a) or (b).

7

WATER-INSOLUBLE SOLIDS<sup>3</sup>—TENTATIVE

Weigh 25 g of the prepared sample, 2(c), into a 400 cc beaker; add 200 cc of H<sub>2</sub>O, cover, heat to boiling, and boil vigorously for 30 min., replacing at intervals the H<sub>2</sub>O lost by evaporation. Prepare about 0.5 g of absorbent cotton or a coarse, qualitative 15 cm filter paper by drying in a water oven and weighing in a flat-bottomed dish provided with a cover. Filter the soln thru the absorbent cotton or filter paper and wash the residue and filter with hot H<sub>2</sub>O until the wash H<sub>2</sub>O is colorless and no longer acid to litmus paper. Transfer the cotton or paper filter containing the insoluble solids to the flat-bottomed dish, dry to constant weight in a water oven, and weigh. The increased weight represents the water-insoluble solids in the sample taken. The filter of absorbent cotton may be prepared by forming it into the shape of the funnel and by means of a wire or glass rod forcing a pledget of the cotton into the stem, but leaving the portion against the sides loose.

8

## SOLUBLE SOLIDS IN FRESH AND CANNED FRUITS, JAMS, MARMALADES AND PRESERVES—TENTATIVE

(Insoluble matter present.)

Proceed as directed under XXXIV, 7. Percentage of soluble solids = % of solids determined by refractometer  $\times \frac{100-b}{100}$ , in which b = water-insoluble solids.

## TOTAL ASH—OFFICIAL

Proceed as directed under XXVII, 8, the temp. of ashing not to exceed 525°, using 25 g of juices, fresh fruits, and canned fruits, and 10 g of jellies, sirups, preserves, jams, marmalades, and dried fruits.

If the ash of the water-soluble portion only is desired, evaporate to dryness 100 cc of the prepared soln, 2(b<sub>1</sub>) or 2(c<sub>1</sub>), and proceed as directed under XXVII, 8.

10

## ALKALINITY OF THE ASH—OFFICIAL

Into the Pt dish containing the ash obtained under 9 introduce a measured excess of 0.1 *N* HCl, warm on a steam bath, cool, add a few drops of methyl orange indicator, and titrate the excess acid with 0.1 *N* NaOH soln. Report the result as alkalinity, the number of cc of 0.1 *N* acid required to neutralize the ash from 100 g of sample, and as alkalinity number, the number of cc of *N* acid required to neutralize 1 g of ash. Reserve the soln for the determination of S in ash.

11

## SULFUR IN ASH—OFFICIAL

(For products containing a basic ash.)

Add 5 cc of HCl (1+2.5) to the soln remaining after the determination of alkalinity of ash under 10 and evaporate to dryness. Heat to 110° for 1 hour to dehydrate any SiO<sub>2</sub>. Take up in 5 cc of the dilute HCl and filter, washing the filter paper well with hot H<sub>2</sub>O. Heat the filtrate to boiling and add dropwise from a buret or pipet 5 cc of 10% BaCl<sub>2</sub> soln. Evaporate to 100 cc and let stand overnight. Filter on a weighed Gooch or Munroe crucible or on a 7 cm ashless filter paper, wash with hot H<sub>2</sub>O until the filtrate is free from chlorides, dry, ignite over a Bunsen burner, and weigh as BaSO<sub>4</sub>. As the quantity of precipitate is small, exercise great care and make the determination in duplicate. Report the result as mg of S per 100 g.

12

## TOTAL SULFUR—TENTATIVE

(For sulfured products and for samples containing little ash or an acidic ash.)

In a casserole as large as can be placed in the electric muffle furnace available, place 1-3 g of MgO [1 g for fruit juices, 3 g for heavily sugared products and for dried fruits—or an equivalent quantity of Mg(NO<sub>3</sub>)<sub>2</sub>], 1 g of powdered sucrose, and 50 cc of HNO<sub>3</sub>. Then add 5-10 g of the prepared sample, 2(a), (b), or (c). Place the same quantities of the reagents in another casserole for a blank. Evaporate on a steam bath to a pasty consistency. Place the casserole in a cold electric muffle and gradually heat to not above dull redness until all N<sub>2</sub>O<sub>4</sub> fumes have been driven off. (All organic matter will have been destroyed.) Cool, dissolve in HCl (1+2.5), and filter. Adjust the acidity so that the soln contains 0.5-1 g of free HCl, heat to boiling, and add dropwise 5 cc of a 10% BaCl<sub>2</sub> soln. Evaporate to 100 cc, allow to stand overnight, filter, wash, ignite, and weigh the BaSO<sub>4</sub>. Correct the result for the BaSO<sub>4</sub> obtained in the blank and report as mg of S per 100 g. The determination should be made in a room free from S fumes.

13

## CHLORINE IN ASH—TENTATIVE

Proceed as directed under XII, 35 and 37.

## POTASSIUM—TENTATIVE

14

## PREPARATION OF SOLUTION

Dissolve the ash in HCl. If an aliquot is desired, filter into a volumetric flask, wash the filter thoroly, and make up to volume. Pipet an aliquot into a beaker, adjust to

a volume of 50–75 cc, heat to boiling, and add a slight excess of  $\text{NH}_4\text{OH}$  and then sufficient saturated  $\text{NH}_4$  oxalate soln to precipitate all the lime and Al present. Continue boiling until the precipitate begins to settle. Filter into a large Pt dish and wash the filter thoroly.

15

## DETERMINATION

Evaporate the soln from 14 nearly to dryness, add 1 cc of  $\text{H}_2\text{SO}_4$  (1+1), evaporate to dryness, and ignite to whiteness. Maintain a full red heat until the residue is perfectly white. Dissolve the residue in hot  $\text{H}_2\text{O}$ , using at least 20 cc for each dg of  $\text{K}_2\text{O}$  present; add a few drops of  $\text{HCl}$  and then an excess of Pt soln, II, 42(b). Evaporate on a water bath to a thick paste, avoiding exposure to  $\text{NH}_3$ . Treat the residue with 90% alcohol. Filter on a dry tared Gooch crucible with an asbestos mat that has been washed thoroly with 90% alcohol and dried at  $100^\circ$  for 30 min. Wash the precipitate thoroly with 90% alcohol, both by decantation and on the crucible mat, and continue the washings after the filtrate is colorless, using about 200 cc of wash solns. Then wash 5 or 6 times with 10 cc portions of  $\text{NH}_4\text{Cl}$  soln, II, 42(a), to remove impurities from the precipitate. Wash again with four or five 10 cc portions of 90% alcohol and dry the precipitate for 30 min. at  $100^\circ$ . Weigh, wash again with several 100 cc portions of 90% alcohol, dry, and reweigh until a constant weight of  $\text{K}_2\text{PtCl}_6$  is obtained. Calculate to  $\text{K}_2\text{O}$ . The precipitate should be completely soluble in  $\text{H}_2\text{O}$ .

## MANGANESE—TENTATIVE

16

## PREPARATION OF SOLUTION

Dissolve the ash in  $\text{HCl}$  (1+2), evaporate to dryness, and heat at  $110^\circ$  for 1 hour to dehydrate any  $\text{SiO}_2$ . Dissolve the residue in  $\text{HCl}$  (1+4) and filter into a volumetric flask. Wash the filter thoroly and make up to volume.

17

## DETERMINATION

To an aliquot of the prepared soln, 16, add sufficient Br water to oxidize any ferrous Fe to the ferric state. Boil off the excess Br. Dilute to 150 cc and heat to boiling. Add sufficient 10%  $\text{NaH}_2\text{PO}_4$  to combine with all the Fe and Al present. Add plenty of bromocresol green indicator, and while the mixture is gently boiling add 10% freshly prepared  $\text{NaOH}$  soln dropwise to the first permanent turbidity or an initial color change in the event no Fe or Al compounds are present. Continue neutralization by slowly adding 20% Na acetate to give a yellow-green color. Fe and Al phosphates are completely precipitated at a pH of 4, at which point bromocresol green indicator is yellow-green.<sup>7</sup> Boil gently for 1–2 min. if any precipitate of Al or Fe phosphate forms. Allow to settle, filter, wash carefully, and discard the precipitate. To the filtrate add 10 cc of the Na acetate and adjust the pH to 4.2–4.4 (indicated by a yellow-green color with bromocresol green indicator) by adding  $\text{HCl}$  (1+5) dropwise. Add sufficient Br water to color the soln distinctly orange, cover with a watch-glass, and boil gently for about 3 min. Take great care to avoid bumping. Allow the mixture to settle, add a little more Br water, and again boil gently for 1–2 min. Again allow to settle, filter, and wash beaker and filter thoroly. The filtrate is reserved for Ca and Mg determinations. Dissolve the hydrated oxide precipitate from the filter into the original beaker with as little soln of  $\text{H}_2\text{O}$  saturated with  $\text{SO}_2$  as possible. Wash the filter paper thoroly with hot  $\text{H}_2\text{O}$ . Boil to remove all odor of  $\text{SO}_2$ , add 10 cc of  $\text{H}_2\text{SO}_4$  and 10–20 cc of  $\text{HNO}_3$ , carefully dilute to 50–75 cc, and heat to boiling, slowly introducing small quantities of  $\text{KIO}_4$  (about 0.05 g)

with a spatula until a maximum color is produced. (About 0.2 g of periodate is sufficient.) Cool, and introduce into a volumetric flask. The amount of Mn in the final dilution for colorimetric comparison should be no more than 1 mg per 50 cc. Compare the color with standards prepared as directed in XII, 14, except to substitute 10 cc of  $\text{HNO}_3$  for the  $\text{Fe}(\text{NO}_3)_3$ . Accurate results may be expected. Report as percentage of  $\text{Mn}_2\text{O}_3$  by multiplying  $\text{KMnO}_4$  by the factor 0.4827.

#### CALCIUM—TENTATIVE

18

##### *Double Precipitation Method*

Evaporate the filtrate from the Mn determination to 100–150 cc. Boil off any Br remaining and adjust the pH to 4.4–4.6 (green to green-blue with bromocresol green indicator) by adding 20% Na acetate. (A pH of 4.4–4.6 is the most favorable for precipitation of Ca oxalate.) Add sufficient saturated Na oxalate soln dropwise to precipitate all the Ca from the boiling soln, and continue to boil until the oxalate begins to settle, or digest for 15 min. on the steam bath. Allow to settle until clear, filter, and wash the precipitate thoroly with hot  $\text{H}_2\text{O}$ . Reserve the filtrate and washings for the Mg determination. Carefully wash the precipitate back into the original beaker, heat, and dissolve the oxalate by adding as little HCl as possible. Reprecipitate the Ca by adding  $\text{NH}_4\text{OH}$  (1+9) soln dropwise until the pH is again 4.4–4.6 (green to green-blue with bromocresol green indicator). Add a slight excess of saturated  $\text{NH}_4$  oxalate soln while still hot. Digest on a steam bath for 1 hour and set aside until the supernatant liquid is clear, preferably overnight. Filter, and wash with hot  $\text{H}_2\text{O}$ . Determine the Ca either gravimetrically or volumetrically by the usual methods. (For small quantities of Ca the gravimetric method is preferred.) Report as  $\text{CaO}$ .

If Mg is not to be determined, precipitate the Ca once from the boiling soln freed from Fe, Al, and Mn with saturated  $\text{NH}_4$  oxalate soln, digest, and determine as directed.

19

##### *Single Precipitation Method*

Evaporate the filtrate and washings from the Mn determination, 17, to 200–250 cc. Add 8–10 drops of bromocresol green indicator and sufficient 20% Na acetate to change the pH to 4.8–5.0 (blue). Cover with a watch-glass and heat to boiling. Precipitate the Ca slowly by adding 3% oxalic acid soln, a drop every 3–5 seconds, until the pH is changed back to 4.4–4.6 (the optimum for Ca oxalate precipitation) as indicated by the appearance of a distinct green shade. A change of color will indicate an excess of oxalic acid—more would develop yellow tints, showing an undesirable displacement of the pH. Boil 1–2 min. and allow to settle until clear. Filter, and wash thoroly with hot  $\text{H}_2\text{O}$ . Determine either gravimetrically or volumetrically as in the double precipitation method.

20

#### MAGNESIUM—TENTATIVE

Add 2–3 drops of HCl to the filtrate and washings from the Ca determination, 18, and evaporate to 75–100 cc. If the quantity of phosphates naturally in the sample, or added for the purpose of precipitating Fe and Al, is insufficient to precipitate all the Mg expected, add more but avoid a large excess. For this purpose neutralize with 10%  $\text{NH}_4\text{OH}$  until a permanent precipitate forms and add sufficient  $\text{NaH}_2\text{PO}_4$  soln to precipitate all Mg present. Dissolve the precipitate by slowly adding 10% HCl dropwise. Use as little HCl as possible to obtain complete soln. (The next step requires considerable care and patience to give accurate results.  $\text{MgHPO}_4$  begins to



precipitate at a pH of 6.7–6.8. This is the critical point.) Heat the soln to gentle boiling and add  $\text{NH}_4\text{OH}$  (1+9) at the rate of 4 drops a min. while maintaining a gentle boil until a crystalline precipitate commences to form. The first precipitate must be crystalline, not gelatinous. If the first precipitate is gelatinous, redissolve it with a little  $\text{HCl}$  and start the precipitation again more slowly. Stirring assists crystallization, but the sides of the beaker should not be scratched. After the crystals have formed in considerable numbers hasten the precipitation. This treatment gives crystalline  $\text{MgHPO}_4$ . Continue the addition of the dilute  $\text{NH}_4\text{OH}$  until the soln is slightly ammoniacal. Allow the mixture to cool slightly, then add  $\frac{1}{2}$  the volume of  $\text{NH}_4\text{OH}$  slowly, and with constant stirring. Let stand until the precipitate has been converted into  $\text{MgNH}_4\text{PO}_4$ , preferably overnight. Filter and wash carefully with the dilute  $\text{NH}_4\text{OH}$ , until all chlorides have been removed. Dry and ignite slowly until all the C is consumed. Cover and ignite intensely. Weigh the white  $\text{Mg}_2\text{P}_2\text{O}_7$  and report as  $\text{MgO}$ .  $\text{Mg}_2\text{P}_2\text{O}_7 \times 0.3621 = \text{MgO}$ . (Ignition of dark colored residues with a drop of 20%  $\text{NH}_4\text{NO}_3$  will often improve the color. If the nitrate is added, use care to avoid spattering.)

## 21

ALCOHOL PRECIPITATE<sup>3</sup>—TENTATIVE

To 100 cc of prepared soln, 2( $b_1$ ) or 2( $c_1$ ), in a beaker, add 4–8 g of sucrose (1 or 2 lumps of cube sugar) if sugar is not already present, and evaporate to a volume of 20–25 cc. If water-insoluble matter separates during evaporation add more sugar. Cool to room temp. and add slowly and with constant stirring 200 cc of 95% alcohol. Allow to stand at least 1 hour, filter on a 15 cm qualitative paper, and wash the precipitate with 95% alcohol. Wash the precipitate back into the original beaker with hot  $\text{H}_2\text{O}$ , rinsing the filter paper thoroly. Evaporate the soln to about 20 cc and add 5 cc of  $\text{HCl}$  (1+2.5). If water-insoluble matter separates, stir well and, if necessary, warm slightly to dissolve. Again precipitate with 200 cc of 95% alcohol, allow to stand 1 hour, and filter thru paper. Wash the precipitate and paper thoroly with 95% alcohol to remove all  $\text{HCl}$ . Rinse the precipitate from the filter paper with hot  $\text{H}_2\text{O}$  into a Pt dish, evaporate to dryness on a steam bath, dry to constant weight in a water oven, and weigh; then ignite and weigh again. The loss in weight is the alcohol precipitate.

As the precipitate in many samples is colorless and almost invisible, care must be exercised that none is lost in the dissolving and transferring operations. If the quantity of the alcohol precipitate, as indicated by its volume in the first precipitation, is not excessive, the second filtration may be made thru a Gooch crucible containing a thin asbestos mat. If the alcohol precipitate is very pure and small in quantity it may not be visible at first. In this case, add a small amount of an electrolyte, like  $\text{NaCl}$ , which will flocculate the alcohol precipitate and render it visible.

## 22

PECTIC ACID<sup>3</sup> (DI-GALACTURONIC ACID)—TENTATIVE

Transfer a 200 cc aliquot of prepared soln, 2( $b_1$ ) or 2( $c_1$ ), to a beaker, add 8–12 g of sucrose (2 or 3 lumps of cube sugar) if the soln does not already contain sugar, and evaporate to about 25 cc. If organic acids are to be determined in the filtrate from the pectin, cool, add 3 cc of  $N$   $\text{H}_2\text{SO}_4$ , and immediately add with constant stirring 200 cc of 95% alcohol, allow the precipitate formed to settle, filter on a 15 cm qualitative paper, and wash with 95% alcohol. If organic acids are not to be determined, omit the addition of the  $\text{H}_2\text{SO}_4$ . Transfer the precipitate to the original beaker with hot  $\text{H}_2\text{O}$ , evaporate to about 40 cc, and cool to 25° or below. If water-insoluble matter separates during evaporation, stir vigorously and, if necessary,

add a few drops of HCl (1+2.5), and warm; then cool again. Add 2-5 cc of 10% NaOH soln (the volume of the precipitate will indicate approximately the quantity to use) diluted with sufficient H<sub>2</sub>O to make a total volume of 50 cc. Allow to stand 15 min., add 40 cc of H<sub>2</sub>O and 10 cc of HCl (1+2.5), and boil for 5 min. Filter, and wash the precipitate of pectic acid with hot H<sub>2</sub>O. This filtration should be rapid and the filtrate clear. If the filtrate is cloudy or of a colloidal nature, reject the determination. Colloidal filtrates are due to insufficient alkali or to saponification at too high a temp., or both. In such cases, repeat the determination, using more alkali and keeping the temp. low. Wash the precipitate of pectic acid back into the beaker, adjust to a volume of 40 cc, cool to below 25°, and repeat the saponification with the dilute NaOH soln, the precipitation with the dilute HCl, and the boiling as above described. Again filter and wash the precipitate of pectic acid with hot H<sub>2</sub>O, but only to the point when a test of the filtrate shows a negligible quantity of acid. (Not more than 500 cc of total filtrate should be necessary.) Wash the pectic acid into a Pt dish and dry on a steam bath and finally in a water oven to constant weight. Weigh, ignite, and weigh again. The loss in weight is pectic acid.

23

## PROTEIN—OFFICIAL

Proceed as directed under II, 21, 23, or 25, using 5 g of jelly or other fruit product containing a large quantity of sugar, or 10 g of juice or fresh fruit, and a larger quantity of the H<sub>2</sub>SO<sub>4</sub> if necessary for complete digestion. Percentage of N  $\times 6.25$  = percentage of protein.

24

## TOTAL ACIDITY—OFFICIAL

Dilute 10 g of prepared juice, 2(a), or 25 cc of prepared soln, 2(b<sub>1</sub>) or 2(c<sub>1</sub>), with recently boiled H<sub>2</sub>O to about 250 cc, or less if the sample is not highly colored. Titrate with 0.1 N alkali, using phenolphthalein indicator. With highly colored products, instead of phenolphthalein soln use azolitmin soln or phenolphthalein powder, XV, 22, on a spot plate. Report the result as cc of 0.1 N alkali per 100 g or 100 cc of the original material.

25

## VOLATILE ACIDS—OFFICIAL

Dissolve 10 g of the sample, dilute to 25 cc, and distil in a current of steam, as directed under XV, 24. 1 cc of 0.1 N alkali = 0.0060 g of acetic acid.

## TOTAL TARTARIC ACID\*

*Bi-tartrate Method—Tentative*

26

## PREPARATION OF SAMPLE

Choose a quantity of sample whose titratable acidity in terms of normal acid does not exceed 3 cc. Designate as "A" the cc of normal alkali required to neutralize the quantity of sample chosen. In no case should the solids content exceed 20 g (200 cc of the sample soln of a jam or jelly).

Adjust the volume of the sample to about 35 cc either by evaporation or by the addition of H<sub>2</sub>O, add 3 cc normal H<sub>2</sub>SO<sub>4</sub>, and heat to 50°.

Pour the adjusted sample into a 250 cc volumetric flask, rinse with 10 cc of hot H<sub>2</sub>O and finally with 95% alcohol, cool, dilute to mark with the alcohol, shake, and filter thru a folded paper (cover funnel with a watch-glass). Pipet 200 cc of the filtrate into a centrifuge bottle.

If the sample contains alcohol, saponification is necessary. Adjust the volume to 35 cc, add "A" + 3 cc of normal KOH, heat to about 60°, and allow to stand

overnight. Add "A" +6 cc of normal  $\text{H}_2\text{SO}_4$  and transfer to a 250 cc volumetric flask, as described above. Filter, and pipet 200 cc of the filtrate into a centrifuge bottle.

27

## DETERMINATION

To the soln in the centrifuge bottle add a quantity of Pb acetate soln equal to "A" +3 cc, or in case saponification was made, "A" +6. Shake vigorously for 2 min., and centrifuge at about 1000 r.p.m. for 15 min. Prepare the Pb acetate soln by dissolving 75 g of normal Pb acetate in  $\text{H}_2\text{O}$  acidulated with 1 cc of glacial acetic acid and diluting to 250 cc with  $\text{H}_2\text{O}$ . Carefully decant the supernatant liquid from the precipitated Pb salts and test with a small quantity of the Pb soln. If a precipitate is formed, return the mixture to the centrifuge bottle, add more Pb soln, shake, and again centrifuge. If the sediment lifts, repeat the centrifuging, increasing the speed and time. Allow to drain thoroly by inverting the bottle for several min. To the material in the centrifuge bottle add 200 cc of 80% alcohol, shake vigorously, and again centrifuge, decant, and drain. To the Pb salts in the centrifuge bottle add about 150 cc of  $\text{H}_2\text{O}$ , shake thoroly, and pass in  $\text{H}_2\text{S}$  to saturation. Transfer to a 250 cc volumetric flask, dilute to mark with  $\text{H}_2\text{O}$ , and filter thru a folded paper. Transfer 200 cc of the clear filtrate to a 400 cc beaker, and evaporate on a gauze to 20 cc over a small flame. Neutralize with normal *potassium* hydroxide, using phenolphthalein indicator, and add 5 drops of the alkali in excess. Add 2 cc of glacial acetic acid and 80 cc of 95% alcohol slowly and with constant stirring. Chill in an ice bath, stir vigorously for 2 min., and place in the refrigerator overnight. Decant the supernatant liquid onto a thin pad of asbestos in a Gooch crucible with a removable bottom, leaving about 25 cc in the beaker. To the contents of the beaker add about 0.3 g of dry purified asbestos. Mix thoroly and wash into the crucible with the cold filtrate. Finally wash the beaker and crucible with 3 portions of 15 cc each of ice cold 80% alcohol, sucking the crucible dry each time. Transfer the pad and precipitate to the original beaker with about 100 cc of hot  $\text{H}_2\text{O}$ , heat almost to boiling, and titrate with 0.1 N alkali, using phenolphthalein indicator. 1 cc of 0.1 N alkali = 0.015 of tartaric acid.

*Racemate Method*<sup>9</sup> (Kling)—Tentative

28

## REAGENTS

(a) *Diammonium-citrate soln.*—Dissolve 29 g of citric acid in about 200 cc of  $\text{H}_2\text{O}$  and carefully neutralize with dilute  $\text{NH}_4\text{OH}$  soln, using methyl red indicator. Add 14.5 g of citric acid, dilute to 1 liter, and filter.

(b) *Ammonium-levo-tartrate soln.*—Dissolve 3.2 g of ammonium-levo-tartrate free of the dextro-modification in  $\text{H}_2\text{O}$ , dilute to 200 cc, and filter. Add 1 cc of formalin as a preservative.

(c) *Calcium acetate soln.*—Dissolve 16 g of  $\text{CaCO}_3$  in 120 cc of glacial acetic acid diluted with  $\text{H}_2\text{O}$ , dilute to 1 liter, and filter.

(d) *Dilute hydrochloric acid soln.*—Dilute 34 cc of  $\text{HCl}$  with  $\text{H}_2\text{O}$  to 1 liter.

(e) *Calcium-sodium acetate soln.*—Dissolve 5 g of  $\text{CaCO}_3$  in 20 g of acetic acid, add 100 g of sodium acetate, dilute to 1 liter, and filter.

(f) *Standard potassium permanganate soln.*—Dissolve 6.9745 g of purest  $\text{KMnO}_4$  in  $\text{H}_2\text{O}$  and dilute to 1 liter. Standardize the soln against a soln of pure tartaric acid of known titer in the same manner as in the final titration. 1 cc of  $\text{KMnO}_4$  soln = nearly 0.005 g of tartaric acid.

(g) *Standard oxalic acid soln.*—Dissolve 13.8793 g of purest oxalic acid in  $\text{H}_2\text{O}$  and dilute to 1 liter. Titrate against the standard  $\text{KMnO}_4$  soln.

## 29

## DETERMINATION

Using the prepared sample, 26, proceed as directed under 27 thru the decomposition of the Pb salts with  $\text{H}_2\text{S}$ , dilution to 250 cc, and filtration. Pipet 200 cc of the clear filtrate into a 400 cc beaker and evaporate to about 100 cc. Add 50 cc of  $\text{H}_2\text{O}$ , 15 cc of the diammonium-citrate soln, 25 cc of the ammonium-levo-tartrate soln, and 20 cc of the calcium acetate soln. Stir vigorously until calcium racemate begins to precipitate and allow to stand overnight at room temp. Decant onto a thin, tightly-tamped pad of asbestos in a Gooch crucible with removable bottom and transfer the precipitate to the Gooch with a portion of the filtrate. Wash the contents of the crucible 5 times with  $\text{H}_2\text{O}$ , filling the crucible about half full and sucking dry each time. Treat the precipitate and mat, after removal from the Gooch, with 20 cc of the dilute  $\text{HCl}$  soln, and wash the crucible thoroly with  $\text{H}_2\text{O}$ . Adjust the volume of the soln to about 150 cc with  $\text{H}_2\text{O}$ , add 50 cc of the calcium-sodium acetate soln, and heat to about  $80^\circ$ . Cool the soln, stir vigorously, and allow to stand at least 4 hours, stirring occasionally. Filter and wash as directed in the first operation. Transfer the pad and precipitate to a casserole with 150 cc of  $\text{H}_2\text{O}$ , add 50 cc of  $\text{H}_2\text{SO}_4$  (1+9), and heat to  $80^\circ$ . Immediately add the standard  $\text{KMnO}_4$  soln until an excess is indicated. Again heat to  $80^\circ$ , add an additional 5 cc of the permanganate soln, and allow to stand about 1 min. After reheating to  $80^\circ$ , immediately add 10 cc of the standard oxalic acid soln and titrate back with the  $\text{KMnO}_4$  soln. The  $\text{KMnO}_4$  soln required for the oxidation  $(\text{cc}) \times 0.005 \div 2 =$  the tartaric acid in the aliquot.

CITRIC ACID<sup>10</sup>—TENTATIVE

## 30

## REAGENTS

*Potassium bromide soln.*—Dissolve 15 g of  $\text{KBr}$  in 40 cc of  $\text{H}_2\text{O}$ .

*Potassium permanganate soln.*—Dissolve 5 g of  $\text{KMnO}_4$  in  $\text{H}_2\text{O}$  and dilute to 100 cc.

*Ferrous sulfate soln.*—Dissolve 40 g of  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  in 100 cc of  $\text{H}_2\text{O}$  containing 1 cc of  $\text{H}_2\text{SO}_4$ .

*Lead acetate soln.*—Dissolve 75 g of normal Pb acetate in  $\text{H}_2\text{O}$ , add 1 cc of glacial acetic acid, and dilute to 250 cc.

## 31

## DETERMINATION

To the prepared soln, 26, in the centrifuge bottle, add a quantity of the Pb acetate soln equal to "A" +3, or in case saponification was made, "A" +6. Shake vigorously for 2 min. and centrifuge at about 1000 r.p.m. for 15 min. Carefully decant the supernatant liquid from the precipitated Pb salts and test with a small quantity of the Pb soln; if a precipitate is formed, return to the centrifuge bottle, add more Pb soln, shake, and again centrifuge. If the sediment lifts, repeat the centrifuging, increasing the speed and time. Allow to drain thoroly by inverting the bottle for several min. To the material in the centrifuge bottle add 200 cc of 80% alcohol, shake vigorously, and again centrifuge, decant, and drain. To the Pb salts in the centrifuge bottle add about 150 cc of  $\text{H}_2\text{O}$ , shake thoroly, and pass in  $\text{H}_2\text{S}$  to saturation. Transfer to a 250 cc volumetric flask, dilute to mark with  $\text{H}_2\text{O}$ , and filter thru a folded paper. Pipet 200 cc of the filtrate into a 500 cc Erlenmeyer flask, and evaporate to about 75 cc. Cool, and add 10 cc of  $\text{H}_2\text{SO}_4$  (1+1) and 5 cc of the potassium bromide soln. Heat the mixture to  $48-50^\circ$ , allow to stand for 5 min., and add 50 cc of the  $\text{KMnO}_4$  soln. Mix, and allow to stand 1 min. Stopper the flask, shake for about 1 min., and allow to stand 3 min. (During this time there should

be a heavy deposit of  $\text{MnO}_2$ ; if necessary, add more  $\text{KMnO}_4$  to assure an excess of the oxidizing agent. If at any time during the oxidation the precipitated  $\text{MnO}_2$  disappears, discard the determination and repeat, using more  $\text{KMnO}_4$ .) Remove the  $\text{MnO}_2$  with the ferrous sulfate soln (about 20 cc), cool to about  $15^\circ$ , stopper the flask, shake vigorously for several min., and place in the refrigerator overnight. Decant the supernatant liquid onto a thin, tightly tamped pad of asbestos in a Gooch crucible (it is important that filtration be completed as quickly as possible). Note volume of filtrate ( $S$  in the formula) and use the filtrate to transfer the precipitate to the crucible. Wash the contents of the crucible at once with 50 cc of ice-cold  $\text{H}_2\text{O}$ . Dry by aspirating with dry air or in a vacuum desiccator and weigh. Remove the pentabromacetone by treating the contents of the crucible with three portions of 20 cc each of alcohol and three portions of 20 cc each of ether. Again dry and weigh. The difference in the two weights represents the weight of pentabromacetone. Calculate the mg of citric acid in the aliquot by the following formula:

$$X = 1.05(0.424P + 0.017S), \text{ in which}$$

$X$  = mg of citric acid in aliquot,

$P$  = weight of pentabromacetone in mg, and

$S$  = volume of filtrate (cc).

For drying the pentabromacetone by aspiration, use the apparatus shown in Fig. 28.

- A, Gooch crucible (28 mm diam.) loosely packed with cotton;
- B, Gooch crucible (35 mm diam.) for pentabromacetone; and
- C, Suction flask (about 500 cc capacity).

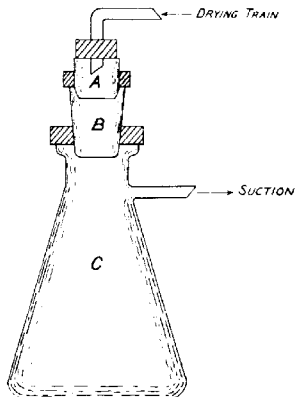


FIG. 28.—APPARATUS FOR DRYING PENTABROMACETONE BY ASPIRATION

Dry the air by passing it thru  $\text{H}_2\text{SO}_4$  and soda-lime, and finally filter thru cotton. Cool the air entering the drying train by passing it thru a spiral condenser cooled with  $\text{H}_2\text{O}$ .

Allow the crucible,  $B$ , containing the pentabromacetone, to remain under suction

for about 1 min. to remove surface moisture before placing in the apparatus. If the air does not pass thru freely, place the crucible in a desiccator for a short time. Maintain a slow uniform flow of air by just "cracking the suction." Dry until the loss in weight does not exceed a few tenths of a mg, making the first weighing after 20 min.

#### LEVO-MALIC ACID<sup>a</sup>—TENTATIVE

32

##### PREPARATION OF SAMPLE

Proceed as directed under 26, omitting the addition of the 3 cc of normal  $H_2SO_4$  to the adjusted sample. In case of saponification add "A" + 3 cc normal  $H_2SO_4$  to the saponified material instead of "A" +  $\beta$  cc.

33

##### REAGENTS

(a) *Lead acetate soln.*—Dissolve 40 g of normal Pb acetate in  $H_2O$ , add 0.5 cc of glacial acetic acid, and dilute to 100 cc.

(b) *Standard tribasic lead acetate soln.*—Prepare the soln from the tribasic lead acetate described below. To 5 g of the salt in a 500 cc Erlenmeyer flask, add 200 cc of distilled  $H_2O$  and shake vigorously. Neutralize 3 cc of normal  $H_2SO_4$ , diluted with 200 cc of  $H_2O$ , with the soln, using methyl red as indicator. Note the volume of the lead soln required. In the determination use 2 cc in excess of this quantity. The solution should be freshly prepared.

(c) *Tribasic lead acetate.*—Dissolve 82 g of normal Pb acetate in 170 cc of distilled  $H_2O$ . Prepare 100 cc of dilute  $NH_4OH$  containing 5.8 g of  $NH_3$  as determined by titration (methyl red). Heat the solns to  $60^\circ$ , mix thoroly, and allow to stand overnight. Shake vigorously to break up the precipitate, and filter on a Büchner funnel; wash once with  $H_2O$  and suck dry, then twice with 95% alcohol, and finally with ether. Allow to dry in air.

34

##### DETERMINATION

To the material in the centrifuge bottle, add about 75 mg of tartaric acid and a quantity of the Pb acetate soln equal to "A," or "A" + 3 cc in case saponification was made, shake vigorously for 2 min. and centrifuge at about 1000 r.p.m. for 15 min. Carefully decant the supernatant liquid from the precipitated Pb salts and test with a small quantity of the Pb acetate soln. If a precipitate is formed, return to the centrifuge bottle, add more Pb acetate, shake, and again centrifuge. If the sediment lifts, repeat the centrifuging, increasing the speed and time. Allow to drain thoroly by inverting the bottle for several min. Add about 200 cc of 80% alcohol, shake vigorously, again centrifuge, decant, and drain. To the Pb salts add about 150 cc of  $H_2O$ , shake vigorously, and pass in a rapid stream of  $H_2S$  to saturation. Stopper the bottle and shake for about 1 min. Transfer to a 250 cc volumetric flask with  $H_2O$ , dilute to mark, shake, and filter thru a folded paper. Pipet 220 cc of the filtrate into a 600 cc beaker and evaporate on a gauze to about 50 cc. Cool, neutralize with normal  $KOH$  (phenolphthalein) and add 5 drops in excess. Add 2 cc of glacial acetic acid and transfer with 95% alcohol to a 250 cc volumetric flask. Add alcohol to mark, shake, and pour into a 500 cc Erlenmeyer flask. Add a small handful of glass beads and cool to  $15^\circ$ . Stopper the flask, shake vigorously for 10 min., and place in the refrigerator for 30 min. Again shake for 10 min. and filter thru a folded paper. Pipet 220 cc of the clear filtrate into a centrifuge bottle, add Pb acetate soln equal to "A" ("A" + 3 cc in the case of saponification), shake vigorously for about 2 min., centrifuge, decant, and drain. Add 200 cc of 80% alcohol, shake, centrifuge,

decant, and drain. Transfer the Pb salts to a 500 cc Erlenmeyer flask with about 175 cc of  $H_2O$ . Add 3 cc of normal  $H_2SO_4$ , heat to boiling, and then add 1 cc of acetic acid soln (5+95) and the quantity of the standard tribasic Pb acetate soln previously determined under 33. Boil the mixture for 5 min., cool to room temp., transfer to a 250 cc volumetric flask with  $H_2O$ , dilute to mark, shake, and pour into a 500 cc Erlenmeyer flask. Add a small handful of glass beads, cool to  $15^\circ$ , shake vigorously for 5 min., and place in the refrigerator for 30 min. Again shake for 5 min. and filter thru a folded paper. Saturate the clear filtrate with  $H_2S$ , shake vigorously, and filter.

**Polarization.**—Evaporate 225 cc of the clear filtrate over a gauze to about 10 cc, neutralize with normal potassium hydroxide (phenolphthalein), make slightly acid with the dilute acetic acid, and evaporate to about 5 cc. Transfer to a 25–27.5 cc Giles flask with  $H_2O$ , dilute to the 27.5 cc mark, shake, and pour into a small Erlenmeyer flask. If a Giles flask is not available, use a 25 cc measuring cylinder, dilute to mark, and add 2.5 cc of  $H_2O$  from a buret. Add a small handful of glass beads and 4 g of powdered uranium acetate, shake vigorously for 10 min., and filter. (As the uranium-malic complex is sensitive to light, while shaking wrap the flask in a towel and protect from light as much as possible during filtration and polarization.) Polarize in a 200 mm tube at  $20^\circ$ , using white light. After filling the tube, release the tension on the glass disks by slightly loosening the caps, and allow to remain at  $20^\circ$  for at least 30 min. before making the readings.

The Ventzke reading  $\times$  the factor 30.1 = the mg of levo-malic acid contained in the portion taken for analysis. (The factor 30.1 is empirical, therefore all directions must be rigidly followed, particularly with respect to dilutions. The substitution of volumetric flasks of different capacities than those specified is not permissible.)

If temp. control is lacking, determine the temp. of the polariscope (lay the thermometer in the trough of the instrument), fill the Giles flask, shake with uranium acetate, and fill the polariscope tube at the temp. of the instrument. Place the tube in the trough of the instrument for 30 min. before making readings.

#### INACTIVE MALIC ACID<sup>12</sup>—TENTATIVE

(The method is empirical, therefore all the directions must be rigidly followed, particularly with respect to dilutions. The substitution of volumetric flasks of different capacities than those specified is not permissible.)

#### 35

##### PREPARATION OF SAMPLE

Subject 2 portions of the sample to the isolation procedure; use one portion for the determination of levo-malic acid (polarization) and the other for total malic acid, levo+inactive (oxidation). Choose a quantity of sample whose titratable acidity does not exceed 150 mg of acid calculated as malic acid. Designate as "A" the cc of normal alkali required to neutralize the quantity of sample chosen. In no case should the solids content exceed 20 g (200 cc of the sample soln of a jam or jelly).

Adjust the volume of the sample to about 35 cc either by evaporation or by the addition of  $H_2O$ , pour into a 250 cc volumetric flask, rinse with 10 cc of hot  $H_2O$  and finally with 95% alcohol, and dilute to mark with the alcohol. Shake, and filter thru a folded paper, draining thoroly and covering the funnel with a watch-glass. Pipet 225 cc of the filtrate to a centrifuge bottle.

If the sample contains alcohol, saponification is necessary. Adjust the volume of the sample to 35 cc, add "A" + 3 cc of normal KOH, heat to about  $60^\circ$ , and allow

to stand overnight. Add "A" + 3 cc of normal  $\text{H}_2\text{SO}_4$  and transfer to a 250 cc volumetric flask, as directed above. Filter, and pipet 225 cc of the filtrate to a centrifuge flask.

## 36

## REAGENTS

Use the reagents described under 33 and in addition—

(d) *Potassium permanganate soln.*—Dissolve 14.5214 g of the purest potassium permanganate in distilled  $\text{H}_2\text{O}$  and dilute to 1 liter. Standardize the soln as follows: Pipet 50 cc of the oxalic acid soln (e) into a 600 cc beaker and add 70 cc of distilled  $\text{H}_2\text{O}$  and 10 cc of  $\text{H}_2\text{SO}_4$  (1+1). Heat to  $80^\circ$ , immediately run in the permanganate soln until a faint pink color is produced, again heat to  $80^\circ$ , and finish the titration. Fifty cc of the permanganate soln should equal 50 cc of the oxalic acid soln; 1 cc of the permanganate soln  $\approx$  5 mg of malic acid (levo or inactive).

(e) *Oxalic acid soln.*—Dissolve 28.7556 g of the purest oxalic acid in distilled  $\text{H}_2\text{O}$  and dilute to 1 liter.

## 37

## DETERMINATION

(a) *Isolation of total malic acid.*—To the soln in the centrifuge bottle add about 25 mg of citric acid and a quantity of the Pb acetate soln equal to "A" (in case saponification is necessary, add "A" + 3 cc), shake vigorously for 2 min., and centrifuge at about 1000 r.p.m. for 15 min. Carefully decant the supernatant liquid from the precipitated Pb salts and test with a small quantity of the Pb acetate soln; if a precipitate is formed, return to the centrifuge bottle, add more Pb acetate, shake, and again centrifuge. If the sediment lifts, repeat the centrifuging, increasing the speed and time. Allow the precipitate to drain thoroly by inverting the bottle for several min. Add 200 cc of 80% alcohol and shake vigorously; again centrifuge, decant, and drain. Add about 150 cc of  $\text{H}_2\text{O}$  to the Pb salts, shake vigorously, and pass in a rapid stream of  $\text{H}_2\text{S}$  to saturation. Stopper the bottle and shake for about 1 min. Transfer the mixture to a 250 cc volumetric flask with  $\text{H}_2\text{O}$ , make to mark, shake, and filter. Pipet 225 cc of the filtrate into a 600 cc beaker, and evaporate to about 100 cc to expel  $\text{H}_2\text{S}$ . Transfer to a 250 cc volumetric flask with  $\text{H}_2\text{O}$ . (The volume in the flask should be about 200 cc.) Add 5 cc of acetic acid (1+9), and the same quantity of Pb acetate soln previously used. Shake vigorously, dilute to mark with  $\text{H}_2\text{O}$ , and filter. Into the clear filtrate pass a rapid stream of  $\text{H}_2\text{S}$  to saturation, stopper the flask, shake vigorously, and filter. Pipet 225 cc of the filtrate into a 600 cc beaker, add about 75 mg of tartaric acid, and evaporate on a gauze to about 50 cc. Cool, neutralize with normal potassium hydroxide (phenolphthalein) and add 5 drops in excess. Add 2 cc of glacial acetic acid and transfer the mixture to a 250 cc volumetric flask with 95% alcohol. Dilute to mark with the alcohol, shake, and pour into a 500 cc Erlenmeyer flask. Add a small handful of glass beads and cool to  $15^\circ$ . Stopper the flask, shake vigorously for 10 min. and place in the refrigerator for 30 min. Again shake for 10 min. and filter thru a folded paper. Adjust the clear filtrate to  $20^\circ$  and pipet 225 cc into a centrifuge bottle. Add Pb acetate soln equal to "A" ("A" + 3 cc in the case of saponification), shake vigorously for about 2 min., centrifuge, decant, and drain. Add 200 cc of 80% alcohol, shake, centrifuge, decant, and drain. Transfer the Pb salts to a 500 cc Erlenmeyer flask with about 175 cc of  $\text{H}_2\text{O}$ . Add 3 cc of normal  $\text{H}_2\text{SO}_4$  and heat to boiling; add 1 cc of acetic acid (5+95) and the quantity of the standard tribasic Pb acetate soln previously determined under 33. Boil the mixture for 5 min., cool to room temp., transfer to a 250 cc volumetric flask with  $\text{H}_2\text{O}$ , make to mark, shake, and pour into a 500 cc Erlenmeyer



flask. Add a small handful of glass beads, cool to about 15°, shake vigorously for 5 min., and place in the refrigerator for 30 min. Again shake for 5 min. and filter thru a folded paper. Saturate the clear filtrate with H<sub>2</sub>S, shake vigorously, and filter. Use one of the two portions for polarization and the other for oxidation.

(b) *Polarization*.—Evaporate 225 cc of the clear soln over a gauze to about 10 cc and proceed as directed under polarization (levo-malic acid), 34.

*Ventzke reading*  $\times$  the factor 10.2 = the mg of levo-malic acid contained in the aliquot ("l" in the formula).

(c) *Oxidation*.—Evaporate 225 cc of the clear soln to about 10 cc to expel the last traces of alcohol, dilute to about 120 cc with H<sub>2</sub>O, and add 10 cc of a 30% NaOH soln and 25 cc of the potassium permanganate soln. Heat to about 80° and place in a boiling water bath for 30 min. Add 25 cc of the oxalic acid soln and 10 cc of H<sub>2</sub>SO<sub>4</sub> (1+1), stirring vigorously. Adjust the temp. to 80°, and titrate to a faint pink color with the permanganate soln. Again heat to 80° and finish the titration. The quantity of permanganate used (cc)  $\times$  5 = the total oxidizable material (as malic acid) present in the aliquot ("t" in the formula).

(d) *Calculation*.—Calculate the inactive malic acid "X" (mg) in the portion taken for analysis by the following formula:

$X = 4(t - 5 - l)$ , in which

t = total oxidizable material (mg) as malic acid;

l = levo-malic acid (mg);

5 = correction factor for the quantity of non-malic material (mg) as malic acid;

4 = factor for reverting inactive malic acid in aliquot back to the quantity of the inactive acid in the portion taken for analysis.

### 38 FREE MINERAL ACIDS—TENTATIVE

Proceed as directed under XXXIII, 80-82.

#### SUCROSE

### 39 By Polarization—Official

Determine by polarizing before and after inversion, as directed under XXXIV, 22, 23 or 27.

### 40 By Reducing Sugars Before and After Inversion—Official

Proceed as directed under XXXIV, 28.

### 41 REDUCING SUGARS OFFICIAL

Proceed as directed under XXXIV, 37, and express the results as invert sugar.

### 42 COMMERCIAL GLUCOSE—OFFICIAL

Proceed as directed under XXXIV, 30.

### 43 DEXTRIN—TENTATIVE

Dissolve 10 g of the sample in a 100 cc flask and add 20 mg of K<sub>2</sub>F and then about  $\frac{1}{4}$  of a cake of compressed yeast. Allow the fermentation to proceed below 25° for 2-3 hours to prevent excessive foaming and then incubate at 27-30° for 5 days. At the end of that time, clarify with basic Pb acetate soln and alumina cream; make up to 100 cc, filter, and polarize in a 200 mm tube. A pure fruit jelly will show a dextro or levo rotation of not more than a few tenths of a degree. If a polariscope having the Ventzke scale is used and a 10% soln is polarized in a 200 mm tube, the number

of degrees read on the sugar scale of the instrument  $\times 0.8755$  = the percentage of dextrin; or the following formula may be used:

$$\text{Percentage of dextrin} = \frac{C \times 100}{198 \times L \times W}, \text{ in which}$$

$C$  = degrees of circular rotation;

$L$  = length of tube in decimeters; and

$W$  = weight of sample in 1 cc.

#### STARCH

44

##### *Qualitative Test—Official*

Dilute a portion of the sample with  $H_2O$ , heat nearly to boiling, add several cc of  $H_2SO_4$  (1+9), and then add 10%  $KMnO_4$  soln until all color is destroyed. Cool, and test with I soln, XXXIII, 28(f). The presence of starch is not necessarily an indication of its addition as an adulterant. It is usually present in small quantity in the apple, and occasionally in other fruits, and unless it is found in the fruit product in considerable quantity its presence may be due to these natural sources.

#### GELATIN<sup>a</sup>

45

##### *Qualitative Test—Tentative*

The presence of gelatin in jellies and jams is shown by the increased content of N. Precipitate a concentrated soln of jelly or jam with 10 volumes of absolute alcohol and determine N in the dried precipitate as directed under II, 21, 23, or 25.

#### AGAR AGAR

46

##### *Detection by Microscopic Examination<sup>14</sup>—Tentative*

Heat the jelly with  $H_2SO_4$  (1+18), add a crystal of  $KMnO_4$ , and allow to settle. If agar agar is present, the sediment will be rich in diatoms, which can be detected by the use of the microscope. The diatoms adhere to the glass and are best obtained by pouring out the liquid, washing the glass with 2 or 3 drops of alcohol, and transferring the alcohol to a microscopic slide by means of a glass rod.

47

##### *Detection by Precipitation—Tentative*

Cover 30 g of the jam or jelly with 270 cc of hot  $H_2O$ , stir until thoroly disintegrated, and boil for 3 min. Filter immediately, while still boiling hot, thru a rapid qualitative filter paper. In the presence of agar agar a precipitate will form upon standing not longer than 24 hours. Filter, wash with cold  $H_2O$ , and dissolve from the paper by means of a very small quantity of boiling  $H_2O$ . Upon chilling this hot  $H_2O$  soln a firm jelly that can be examined by the touch will be formed. This method will detect 0.2% of agar agar with certainty if the proportions of jam or jelly and  $H_2O$  are strictly observed.

48

##### ADDED WATER IN GRAPE JUICE<sup>a</sup>—TENTATIVE

(Applicable to white juices only.)

Measure about 50 cc of the *filtered* juice into a 2 oz tincture bottle containing a number of short pieces of glass rods. Add about 1 g of finely powdered purest potassium acid tartrate, cool to 25°, and shake 1 hour at this temp. (There should be undissolved bitartrate in the juice; if there is not, repeat the operation, using more

of the salt.) *Immediately* filter the juice and titrate 10 cc of the filtrate with 0.1 *N* alkali, using phenolphthalein indicator. In the same manner titrate 10 cc of the original filtered juice. An increase in the titer of the treated sample is an index of added H<sub>2</sub>O. The two titrations should be made side by side in order to obtain the same shade of pink.

For control of temp. during the saturation period the following procedure is suggested: Immerse the tightly corked tincture bottle, neck down, into a pint Mason jar filled top-full with H<sub>2</sub>O at 25°. Adjust the Mason jar cover, immerse the jar in a pail of H<sub>2</sub>O of 25° and maintain this temp. for 30 min. Remove the jar from the H<sub>2</sub>O and immediately wrap in 3 sheets of heavy wrapping paper, making each wrapping separately. Place the system in a shaker and shake for 1 hour. Ascertain the temp (*t*° in the formula) of the H<sub>2</sub>O in the jar. Determine the titers of the treated and untreated juices as directed above and calculate the volume % of added sugar soln (H<sub>2</sub>O) by the following formula:

$$W = \frac{0.0188(b-a) - 0.095 - 0.025\left(\frac{t^\circ - 25}{2}\right)}{0.006}, \text{ in which}$$

*W* = Vol. % added H<sub>2</sub>O (20% sugar soln),

*b* = Acidity of treated juice, cc 0.1 *N* alkali per 100 cc,

*a* = Acidity of original juice, cc 0.1 *N* alkali per 100 cc, and

*t*° = Temperature of H<sub>2</sub>O in Mason jar after shaking.

Pure factory juices examined by this method show a small quantity of added H<sub>2</sub>O (1-3%).

## 49

## METALS

Proceed as directed under XXIX.

## 50

## PRESERVATIVES

Proceed as directed under XXXII.

## 51

## COLORING MATTERS

Proceed as directed under XXI.

## 52

## SWEETENING SUBSTITUTES

Proceed as directed under XXXII, 13, 14, 38, 39.

## SELECTED REFERENCES

- <sup>1</sup> J. Assoc. Official Agr. Chem., 17, 66 (1934).
- <sup>2</sup> Ibid., 18, 80 (1935).
- <sup>3</sup> Ibid., 6, 34 (1922).
- <sup>4</sup> Ibid., 15, 76 (1932).
- <sup>5</sup> Ibid., 6, 37 (1922).
- <sup>6</sup> Ibid., 11, 216 (1928); 15, 76 (1932).
- <sup>7</sup> Clark, The Determination of Hydrogen Ions, 3rd ed. (1928).
- <sup>8</sup> J. Assoc. Official Agr. Chem., 12, 366 (1929).
- <sup>9</sup> Bull. soc. chim., 7, 567 (1910); 11, 886 (1912); J. Assoc. Official Agr. Chem., 8, 638 (1925); 13, 103 (1930).
- <sup>10</sup> J. Assoc. Official Agr. Chem., 13, 99 (1930); 14, 64 (1931).
- <sup>11</sup> Ibid., 15, 648 (1932).
- <sup>12</sup> Ibid., 16, 281 (1933).
- <sup>13</sup> Chem. Ztg., 19, 552 (1895).
- <sup>14</sup> Z. angew. Mikrosk., 2, 260 (1896); Z. Nahr. Genussm., 21, 185 (1911).
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## XXVII. GRAIN AND STOCK FEEDS

### 1 PREPARATION OF SAMPLE—OFFICIAL

Grind the sample to pass thru a sieve having circular openings  $1/25$  in. (1 mm) in diameter and mix thoroly. If the sample cannot be ground, reduce it to as fine a condition as possible.

#### MOISTURE<sup>1</sup>

### 2 I. Drying with Heat—Official

Dry to constant weight at  $95-100^{\circ}$  under a pressure not to exceed 100 mm of Hg (approximately 5 hours), a quantity of the substance representing about 2 g of dry material. Use a covered Al dish at least 50 mm in diameter and not exceeding 40 mm deep. If the substance is contained in a glass vessel, the latter should not come in contact with the boiling  $H_2O$ . Report the loss in weight as moisture.

### II. By Distillation with Toluene—Official

### 3 APPARATUS

A 250 cc distilling flask of Pyrex or other resistant glass connected by means of a "distilling tube receiver" to a 20-in., sealed-in, straight-tube Liebig condenser with delivery tube not over  $5/16$  in. in diameter in the manner shown in Fig. 29. The distilling tube receiver is of the dimensions shown and is made by attaching a proper side tube to the calibrated section of a 5 cc Mohr pipet and sealing the outlet. The tube is calibrated in lengths of a cc by distilling known quantities of  $H_2O$  into the graduated column, and the column of  $H_2O$  may be read to hundredths with reasonable accuracy. Clean the tube and condenser with  $Cr_2O_3-H_2SO_4$  mixture, rinse thoroly with  $H_2O$ , then with alcohol, and dry in an oven to prevent an undue quantity of  $H_2O$  adhering to the inner surfaces during the determination.

### 4

#### DETERMINATION

If the sample is likely to bump, add enough dry sand to cover the bottom of the flask. Add sufficient toluene to cover the sample completely (about 75 cc). Weigh and introduce into the toluene sufficient sample to give 2-5 cc of  $H_2O$  and connect the apparatus as shown in Fig. 29. Fill the receiving tube with toluene, pouring it thru the top of the condenser. Bring to a boil and distil slowly, about 2 drops per second, until most of the  $H_2O$  has passed over; then increase the rate of distillation to about 4 drops per second. When all the  $H_2O$  is apparently over, wash down the condenser by pouring toluene in at the top, continuing the distillation a short time to ascertain whether any more  $H_2O$  will distil over; if it does, repeat the washing down process. If any  $H_2O$  remains in the condenser, remove it by brushing down with a tube brush attached to a Cu wire and saturated with toluene, washing down the condenser at

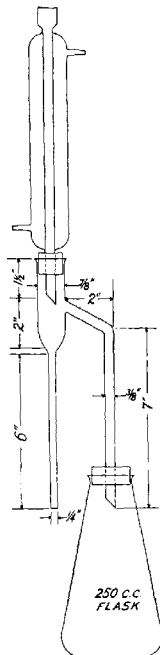


FIG. 29.—APPARATUS USED IN METHOD II. FOR THE DETERMINATION OF MOISTURE

the same time. The entire process is usually completed within an hour. Allow the receiving tube to come to room temp. If any drops adhere to the sides of the tube, force them down by means of a rubber band wrapped around a Cu wire. Read the volume of  $\text{H}_2\text{O}$  and calculate to percentage.

### III. Drying without Heat over Sulfuric Acid<sup>2</sup>—Official

5

#### REAGENT

*Sulfuric acid*—Boil  $\text{H}_2\text{SO}_4$  in a large Kjeldahl flask for 4 hours, close the mouth of the flask with a stopper carrying a  $\text{CaCl}_2$  tube, and cool.

6

#### DETERMINATION

Weigh a suitable quantity of the sample (2–5 g) into a metal dish 5–10 cm in diameter and provided with a tightly fitted cover. If subsequent fat determinations are to be made, fat extraction cones may be used. Substances that dry down to horn-like material should be mixed with fat-free cotton or other suitable material. Place 200 cc of the fresh  $\text{H}_2\text{SO}_4$  in a strong, tight vacuum desiccator. Then place the dish, uncovered, in the desiccator and exhaust by means of a vacuum pump to a pressure of not more than 10 mm of Hg.

If a pump is not available, place 10 cc of ether in a small beaker in the desiccator and exhaust with a water filter pump. Between the pump and the desiccator interpose an empty bottle next to the desiccator and a bottle of  $\text{H}_2\text{O}$  next to the pump. Draw the air from the desiccator thru the  $\text{H}_2\text{O}$  and turn the desiccator stopcock the instant the  $\text{H}_2\text{O}$  begins to rise in the tube leading from the empty bottle.

Gently rotate the desiccator 4 or 5 times during the first 12 hours. At the end of 24 hours open the desiccator, causing the incoming air to bubble thru  $\text{H}_2\text{SO}_4$ , place the cover on the dish, and make the first weighing. After weighing place the sample in a desiccator containing fresh  $\text{H}_2\text{SO}_4$  and exhaust as before. Rotate the desiccator several times during the interval and weigh again after a suitable period of drying. Repeat this process until the weight is constant.

7

### IV. Electric Air-Oven Method<sup>3</sup>—Official

(Not intended for use when a subsequent fat determination is to be made on the same sample.)

Regulate an electric air oven to  $135^\circ \pm 2^\circ$ . Using low, covered Al dishes, 2, weigh approximately 2 g of the sample into each dish and shake until the contents are evenly distributed. With the covers removed, place the dishes and covers in the oven as quickly as possible and dry the samples for 2 hours. After placing the covers on the dishes transfer them to a desiccator to cool. Weigh, and calculate the loss in weight as moisture.

8

#### ASH—OFFICIAL

Weigh a quantity of the substance representing about 2 g of dry material and burn at a low heat, not exceeding dull redness, until free from C. If a C-free ash cannot be obtained in this manner, exhaust the charred mass with hot  $\text{H}_2\text{O}$ , collect the insoluble residue on an ashless filter, and burn the filter and contents to a white or nearly white ash. Add the filtrate, evaporate to dryness, and heat at dull redness until the ash is white or grayish white. Cool in a desiccator and weigh.

9

#### CRUDE PROTEIN—OFFICIAL

Determine N as directed under II, 21, 23, or 25, and multiply the result by 6.25.

QUALITATIVE TESTS FOR PROTEINS<sup>4</sup>—OFFICIAL, FIRST ACTION*Biuret Test*

(Unreliable in the presence of glycerol.)

10

## REAGENT

Add slowly with stirring 25 cc of a 3% soln of  $\text{CuSO}_4$  to 1 liter of 10%  $\text{NaOH}$ . If it is necessary to filter the reagent, use glass wool.

11

## DETERMINATION

To 2 or 3 cc of the protein soln add, with shaking, a few drops of the reagent. If the characteristic pink or violet color does not develop quickly, allow the soln to stand for 15 or 20 min. In the presence of  $(\text{NH}_4)_2\text{SO}_4$  the addition of  $\text{NaOH}$  is necessary.

(a) *Osborne's modification*.—This modification of the biuret test greatly increases its delicacy. Make the test as described above. Then add 10–20 drops of 95% ethyl alcohol and a piece of solid  $\text{NaOH}$  (about 5 g). The alkali "salts out" the small quantity of alcohol, which carries with it the color present, and in this way the presence of small quantities of protein can be detected.

The biuret test is dependent on the peptide grouping,  $-\text{HN} \cdot \text{CO} \cdot \text{NH}-$ , and therefore is given by all proteins. It is also given by certain other compounds containing similar groupings, such as biuret,  $\text{H}_2\text{N} \cdot \text{CO} \cdot \text{NH} \cdot \text{CO} \cdot \text{NH}_2$ , and malonamide,  $\text{H}_2\text{N} \cdot \text{CO} \cdot \text{CH}_2 \cdot \text{CO} \cdot \text{NH}_2$ . Compounds containing one  $-\text{CO} \cdot \text{NH}_2$  and one  $-\text{CSNH}_2$ ,  $-\text{C}(\text{NH})\text{NH}_2$  or  $-\text{CH}_2\text{NH}_2$  similarly joined will also respond to this test.

*Millon's Test*

(Given by all aromatic substances, such as phenol and salicylic acid, which contain a benzene nucleus with a substituted hydroxyl group. In proteins this grouping is furnished by the amino acid tyrosine.)

12

## REAGENT

Dissolve, by gently warming, one part by weight of  $\text{Hg}$  in two parts by weight of  $\text{HNO}_3$ , sp. gr. 1.42. Dilute the soln with two volumes of  $\text{H}_2\text{O}$ . Allow the mixture to stand overnight and decant the supernatant liquid. The soln contains  $\text{Hg}(\text{NO}_3)_2$  and  $\text{HgNO}_3$ ,  $\text{HNO}_3$ , and some  $\text{HNO}_2$ .

13

## DETERMINATION

Add a few drops of the reagent to 4 or 5 cc of the protein soln in a test tube. Warm gently by immersing for a few minutes in hot  $\text{H}_2\text{O}$ . A pink or a red color slowly develops and a precipitate usually forms. If the substance is a solid, suspend in 3 or 4 cc of  $\text{H}_2\text{O}$  and treat as directed above. Alkaline solns should first be neutralized to avoid precipitation of  $\text{HgO}$ .

*Glyoxylic Acid Test (Hopkins-Cole)*

14

## REAGENT

Add sufficient  $\text{H}_2\text{O}$  to cover liberally 10 g of powdered  $\text{Mg}$  in a large Erlenmeyer flask. Add 250 cc of a cold saturated soln of oxalic acid, keeping the flask cool under the water tap during the addition of the acid. After the reaction is over shake the mixture and filter. Acidify the filtrate with acetic acid and make the volume up to 1 liter with distilled  $\text{H}_2\text{O}$ .

## 15

## DETERMINATION

To 1 or 2 cc of the protein soln in a test tube add 3 cc of the reagent and mix thoroly. By means of a pipet allow the mixture to flow gently down the side of a second test tube (slightly inclined) containing 5 cc of  $\text{H}_2\text{SO}_4$ . A reddish-violet color forms at the junction of the fluids, owing to the presence of tryptophane in the protein.

## 16

*Adamkiewicz Test*

Proceed as directed under 15, except to use glacial acetic acid instead of a prepared soln of glyoxylic acid. The color reaction depends on the presence of traces of glyoxylic acid formed from the glacial acetic acid.

## 17

*Xanthoproteic Test*

Add about 1 cc of  $\text{HNO}_3$  to 3 cc of the protein soln. A white precipitate forms, which on boiling assumes a yellow color and may dissolve to give a yellow soln. Cool, and make slightly alkaline by the careful addition of 30%  $\text{NaOH}$ . The color changes to deep orange. The color development depends on the formation of nitro derivatives attached to the benzene nucleus, and in proteins is referable primarily to the amino acids tyrosine and phenylalanine.

## ALBUMINOID NITROGEN - OFFICIAL

## 18

## REAGENT

*Cupric hydroxide*.—Dissolve 100 g of  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  in 5 liters of  $\text{H}_2\text{O}$ ; add 2.5 cc of glycerol, and then add 10%  $\text{NaOH}$  soln until the liquid is slightly alkaline; filter; rub the precipitate in a mortar with  $\text{H}_2\text{O}$  containing 5 cc of glycerol per liter; and wash by decantation or filtration until the washings are no longer alkaline. Again rub the precipitate in a mortar with  $\text{H}_2\text{O}$  containing 10% of glycerol, thus preparing a uniform gelatinous mass that can be measured with a pipet. Determine approximately the quantity of  $\text{Cu}(\text{OH})_2$  in 5 cc by diluting to 50 cc with  $\text{H}_2\text{O}$ , filtering, washing, igniting, and weighing as  $\text{CuO}$ .

## 19

## DETERMINATION

Place 0.7 g of the sample in a beaker, add 100 cc of  $\text{H}_2\text{O}$ , and heat to boiling; or, in case of substances rich in starch, heat on a steam bath for 10 min., add a quantity of the reagent that contains about 0.5 g of the  $\text{Cu}(\text{OH})_2$ , stir thoroly, filter when cold, wash with cold  $\text{H}_2\text{O}$ , and without removing the precipitate from the filter determine the N as directed under II, 21, 23 or 25, adding sufficient  $\text{K}_2\text{S}$  or  $\text{Na}_2\text{S}$  soln, II, 19(b), to precipitate all the Cu and Hg. The filter paper used must be practically free from N. If the material (such as seeds, seed residue, or oil cake) is rich in alkaline phosphates, add 1-2 cc of a 10% soln of  $\text{NH}_3$ -free soda alum to decompose the alkaline phosphates, then the  $\text{Cu}(\text{OH})_2$ , and mix well by stirring. If this is not done, Cu phosphate and free alkali may be formed and the protein-copper precipitate partially dissolved in the alkaline liquid.

## 20

## AMIDO NITROGEN - OFFICIAL

Subtract the percentage of albuminoid N from the percentage of total N to obtain the amido N.

## CRUDE FAT OR ETHER EXTRACT

*Direct Method—Official*

21

## REAGENT

*Anhydrous ether.*—Wash commercial ether with 2 or 3 successive portions of  $H_2O$ , add solid  $NaOH$  or  $KOH$ , and let stand until most of the  $H_2O$  has been abstracted from the ether. Decant into a dry bottle, add small pieces of carefully cleaned metallic  $Na$ , and let stand until there is no further evolution of  $H$  gas. Keep the ether, thus dehydrated, over metallic  $Na$  in loosely stoppered bottles.

22

## DETERMINATION

Large quantities of soluble carbohydrates may interfere with the complete extraction of the fat. In such cases extract with  $H_2O$  before proceeding with the determination. Extract about 2 g of the sample, dried as directed under 2, 6, or 7, with the anhydrous ether for 16 hours. Dry the extract at the temp. of boiling  $H_2O$  for 30 min., cool in a desiccator, and weigh; continue, at 30 min. intervals, this alternate drying and weighing until the weight is constant. For most feeds a period of 1–1.5 hours is required.

23

*Indirect Method—Official*

Determine moisture as directed under 2, 6, or 7; then extract the dried substance for 16 hours as directed under 22, and dry again. Report the loss in weight as ether extract.

## FAT IN DRIED MILK PRODUCTS

24

*Modified Roese-Gottlieb Method<sup>2</sup>*

Weigh 1 g of well-mixed milk powder and transfer immediately into a dry Mojonnier extraction flask or a dry Röhrig tube. Add 8.5 cc of warm  $H_2O$ , cork, and shake vigorously until dissolved, warming slightly if necessary to room temp. Add 1.5 cc of  $NH_4OH$  and shake thoroly; add 10 cc of 95% ethyl alcohol and shake thoroly; add 25 cc of ethyl ether, cork, and shake thoroly; and finally add 25 cc of petroleum ether and shake as before. Allow the ether layer to separate by leaving the flask or tube at rest for 20 min. or until the upper liquid is practically clear. Draw off as much as possible of the ether fat soln in a flask or Al dish. Evaporate on the hot plate or steam bath at a temp. that effects complete evaporation, but not so high that spattering or vigorous boiling will result. To the residue in the flask or tube add 4 cc of 95% ethyl alcohol and mix thoroly without inserting the stopper. Add 15 cc of ethyl ether and shake thoroly; add 15 cc of petroleum ether and again shake thoroly. Let stand and separate the ether layer as before, drawing it off into the same flask or dish and evaporating the ether.

Make a third extraction in exactly the same manner as the second, omitting the addition of alcohol. If necessary, carefully pour a few cc of distilled  $H_2O$  down the side of the tube to raise the level of the aqueous layer, so the ethers may be completely poured off. (At no time should any of the aqueous layer be allowed to run into the dish.)

After the ether is entirely evaporated, place the dish in a Mojonnier oven for 5 min. with the temp. at exactly  $135^\circ$ , or in a boiling water oven for 30 min., or longer if required to bring it to constant weight.

Remove the fat completely with petroleum ether and dry the residue; weigh, and



deduct from the total weight. The loss in weight is the percentage of fat. Finally, correct this weight by a blank determination on the reagents used.

NOTES.—The time required for each shaking after addition of the first portion of alcohol and subsequent additions of either ethyl or petroleum ether should be not less than 30 seconds, and the shaking should be very vigorous.

Each time after drawing off the ether layer the lip of the extraction flask or the spigot should be rinsed with petroleum ether, and the rinsings allowed to run into the Al dish.

The cork should be washed down at least once with petroleum ether.

Petroleum ether should have a boiling point below 60°, and both petroleum and ethyl ether should be free from residue on evaporation.

The official method, XXII, 19, requires the use of a small quick-acting filter for drawing off the ether layer and washing the filter with petroleum ether. However, if care is used in making the separation, the use of the filter is not absolutely necessary.

#### CRUDE FIBER—OFFICIAL

#### 25

##### REAGENTS

(a) *Sulfuric acid soln.*—Contains 1.25 g of  $\text{H}_2\text{SO}_4$  per 100 cc.

(b) *Sodium hydroxide soln.*—Contains 1.25 g of NaOH per 100 cc, free, or nearly so, from  $\text{Na}_2\text{CO}_3$ .

The strength of these solns must be accurately checked by titration.

(c) *Asbestos.*—Digest on a steam bath or at an equivalent temp. for at least 8 hours with an approximately 5% NaOH soln and thoroly wash with hot  $\text{H}_2\text{O}$ ; then digest in a similar manner for 8 hours with HCl (1+3) and again wash thoroly with hot  $\text{H}_2\text{O}$ . Dry, and ignite at bright red heat.

#### 26

##### APPARATUS

(a) *Condenser.*—Use a condenser that will maintain a constant volume of soln throught the process of digestion.

(b) *Digestion flasks.*—Use digestion flasks of such size and shape that the soln will be not less than 1 in. nor more than 1.5 in. in depth. A 700–750 cc Erlenmeyer flask is recommended.

(c) *Filtering cloth.*—Use filtering cloth of such character that no appreciable solid matter passes thru when filtering is rapid. Butchers linen or dress linen with about 45 threads to the inch or No. 40 filtering cloth made by the National Filter Cloth and Weaving Company, or its equivalent, may be used.

#### 27

##### DETERMINATION

Extract 2 g of the dry material with ordinary ether, or use the residue from the ether extract determination (22 or 23), and transfer the residue, together with about 0.5 g of asbestos, to the digestion flask. (If the residue from the ether extract is used and the proper quantity of asbestos has already been added, further addition is unnecessary.) Add 200 cc of the boiling  $\text{H}_2\text{SO}_4$  soln, immediately connect with the condenser, and heat. (It is essential that the contents of the flask come to boiling within 1 min. and that the boiling continue briskly for exactly 30 min.) Rotate the flask about every 5 min. in order to mix the charge thoroly. Take care to keep the material from remaining on the sides of the flask out of contact with the soln. (A blast of air conducted into the flask will serve to reduce frothing of the liquid.) At the expiration of 30 min. remove the flask, immediately filter thru linen in a fluted funnel, and wash with boiling  $\text{H}_2\text{O}$  until the washings are no longer acid. Bring a quantity of the NaOH soln to boiling and keep at this temp. under a reflux

condenser until used. Wash the charge and asbestos back into the flask with 200 cc of the boiling NaOH soln, using a wash bottle marked to deliver 200 cc. (The boiling NaOH soln is conveniently transferred to the 200 cc wash bottle by means of a bent tube thru which the liquid is forced by blowing into a tube connected with the top of the reflux condenser attached to the NaOH flask.) Then connect the flask with the reflux condenser and boil for exactly 30 min. The boiling with the alkali should be so timed that the contents of the different flasks will reach the boiling point approximately 3 min. apart, which permits sufficient time for filtration. At the expiration of 30 min. remove the flask and immediately filter thru a Gooch prepared with an asbestos mat, thru an alundum crucible, or thru the filtering cloth in a fluted funnel. If the filtering cloth is used, thoroly wash the residue with boiling H<sub>2</sub>O and then transfer it to a Gooch crucible prepared with a thin but close layer of ignited asbestos. After thoro washing with boiling H<sub>2</sub>O, wash with about 15 cc of 95% alcohol. Dry the crucible and contents at 110° to constant weight. Cool in an efficient desiccator and weigh. Incinerate the contents of the crucible in an electric muffle or over a Meker burner at a dull red heat until the carbonaceous matter has been consumed (about 20 min.). Cool in a desiccator and weigh. Report the loss in weight as crude fiber.

## 28

## PREPARATION OF SOLUTION FOR SUGARS—OFFICIAL

Place 10 g of the material in a 250 cc volumetric flask. If the substance has an acid reaction, add 1–3 g of CaCO<sub>3</sub> to neutralize the acidity. Add 125 cc of 50% alcohol by volume, mix thoroly, and boil on a steam bath for 1 hour, using a small funnel in the neck of the flask to condense the vapor. Cool, and allow the mixture to stand several hours, preferably overnight. Make up to volume with neutral 95% alcohol, mix thoroly, and allow to settle. Pipet 200 cc of the supernatant soln into a beaker and evaporate on a steam bath to a volume of 20–30 cc. Do not evaporate to dryness. A little alcohol in the residue does no harm. Transfer to a 100 cc volumetric flask and rinse the beaker thoroly with H<sub>2</sub>O, adding the rinsings to the contents of the flask. Add enough saturated neutral Pb acetate soln (approximately 2 cc) to produce a flocculent precipitate, shake thoroly, and allow to stand 15 min. Dilute to the mark with H<sub>2</sub>O, mix thoroly, and filter thru a dry filter. Add sufficient anhydrous Na<sub>2</sub>CO<sub>3</sub> or K oxalate to the filtrate to precipitate all the Pb, again filter thru a dry paper, and test the filtrate with a little anhydrous Na<sub>2</sub>CO<sub>3</sub> or K oxalate to make sure that all the Pb has been removed.

## 29

## REDUCING SUGARS—OFFICIAL

Proceed as directed under XXXIV, 37 or 48, using 25 cc of the soln (representing 2 g of the sample) prepared as directed under 28. Express the results as dextrose or invert sugar.

## 30

## SUCROSE—OFFICIAL

Introduce 50 cc of the soln prepared as directed under 28 into a 100 cc volumetric flask, add a piece of litmus paper, neutralize with HCl, add 5 cc of HCl, and allow the inversion to proceed at room temp. as directed under XXXIV, 23(c). When inversion is complete, transfer the soln to a beaker, neutralize with Na<sub>2</sub>CO<sub>3</sub>, return the soln to the 100 cc flask, dilute to the mark with H<sub>2</sub>O, filter if necessary, and determine reducing sugars in 50 cc of the soln (representing 2 g of the sample) as directed under 29. Calculate the results as invert sugar. Subtract the percentage of reducing sugars before inversion from the percentage of total sugar after inver-

sion, both calculated as invert sugar, and multiply the difference by 0.95 to obtain the percentage of sucrose present.

Because the insoluble material of grain or cattle food occupies some space in the flask as originally made up, it is necessary to correct for this volume. To obtain the true quantity of sugars present multiply all results by the factor 0.97, as results of a large number of determinations on various materials have shown the average volume of 10 g of material to be 7.5 cc.

#### STARCH

31

##### *I. Direct Acid Hydrolysis—Official*

(Intended only for such materials as raw starch, potatoes, etc., including as starch the pentosans and other carbohydrate bodies that undergo hydrolysis and are converted into reducing sugars on boiling with HCl.)

Stir a weighed quantity of the sample, representing 2.5–3 g of the dry material, in a beaker with 50 cc of cold H<sub>2</sub>O for an hour. Transfer to a filter and wash with 250 cc of cold H<sub>2</sub>O. Heat the insoluble residue for 2.5 hours with 200 cc of H<sub>2</sub>O and 20 cc of HCl (sp. gr. 1.125) in a flask provided with a reflux condenser. Cool, and nearly neutralize with NaOH. Complete the volume to 250 cc, filter, and determine the dextrose in an aliquot of the filtrate as directed under XXXIV, 46 or 48. Weight of dextrose obtained  $\times 0.90$  = weight of starch.<sup>5</sup>

##### *II. Diastase Method with Subsequent Acid Hydrolysis—Official*

32

#### REAGENT

*Malt extract.*—Use clean, new barley malt of known efficacy and grind only as needed. Grind well, but not so fine that filtration will be greatly retarded. Prepare an infusion of the freshly ground malt just before it is to be used. For every 80 cc of the malt extract required digest 5 g of the ground malt with 100 cc of H<sub>2</sub>O, at room temp., for 2 hours, or for 20 min. if the mixture can be stirred by an electric mixer. Filter to obtain a clear extract (it may be necessary to return the first portions of the filtrate to the filter). Mix the infusion well.

33

#### DETERMINATION

Extract a quantity of the substance (ground to an impalpable powder and representing 4–5 g of the dry material) on a hardened filter with 5 successive portions of 10 cc of ether; wash with 150 cc of alcohol, 10% by volume, and then with a few cc of 95% alcohol. Place the residue in a beaker with 50 cc of H<sub>2</sub>O, immerse the beaker in boiling H<sub>2</sub>O, and stir constantly for 15 min., or until all the starch is gelatinized; cool to 55°, add 20 cc of the malt extract, and maintain at this temp. for an hour. Heat again to boiling for a few min., cool to 55°, add 20 cc of the malt extract, and maintain at this temp. for an hour, or until the residue treated with 1 soln shows no blue color upon microscopical examination. Cool, make up directly to 250 cc, and filter. Place 200 cc of the filtrate in a flask, add 20 cc of HCl (sp. gr. 1.125), connect with a reflux condenser, and heat in a boiling water bath for 2.5 hours. Cool, nearly neutralize with 10% NaOH soln, finish the neutralization with Na<sub>2</sub>CO<sub>3</sub> soln, and dilute to 500 cc. Mix the soln thoroly, pour thru a dry filter, and determine the dextrose in an aliquot as directed under XXXIV, 46 or 48. Conduct a blank determination upon the same volume of the malt extract as used with the sample and correct the weight of dextrose accordingly. Weight of dextrose obtained  $\times 0.90$  = weight of starch.

*III. In the Presence of Interfering Polysaccharides<sup>2</sup>—Official*

Weigh 2–6 g (charges of 4 g for linseed meal, or 3 g for dried apple pomace, have been found to be satisfactory) of the well-mixed sample, prepared to pass freely thru a sieve not less than 40 mesh to the inch, using the smaller charges in the case of materials containing much gel-forming substance. (The weight of starch in the charge must not exceed 1.5 g.) Transfer to a dry 12.5–15 cm close-textured rapid filtering paper in a glass funnel and extract with 5 successive portions of ether, taking for each portion more than enough to cover the charge and using a cover-glass to retard evaporation. After completing the ether extraction, allow the ether to evaporate and then extract the charge with 300 cc of dilute alcohol. The concentration of the alcohol may be varied somewhat to suit the material under examination. For linseed meal use 35% alcohol (by volume) and for dried apple pomace use 25% alcohol. Follow this with several filterfuls of 95% alcohol and finish the leaching operations with a second ether extraction. Conduct also a control determination, preferably in duplicate, using a filter paper extracted with alcohol and the same quantity of H<sub>2</sub>O and malt extract as in the determination. (It is convenient to let the charge stand overnight at this point to allow the ether and alcohol to evaporate, as alcohol must be eliminated before starting the digestion with malt; or the charge may be dried at approximately 75° until the alcohol has been eliminated.)

Transfer as much of the dry material as possible from the filter paper into a glass mortar and pulverize all lumps. Transfer both filter paper and sample to a 500 cc volumetric flask, add 20–30 cc of H<sub>2</sub>O, and thoroly wet the material by vigorous shaking.

Should more cold H<sub>2</sub>O be needed to make the material more fluid, calculate the quantity of hot H<sub>2</sub>O to be added accordingly, so that the total volume allowing for 40 cc of malt soln will not exceed 200 cc. Let stand a few minutes, add 100 cc of actively boiling H<sub>2</sub>O, and thoroly gelatinize in a boiling water bath.

Cool to 50° or lower, add 20 cc of malt extract, 32, to controls as well as to charges, and place the flasks in a temp.-controlled water bath. Keeping the mash thoroly mixed, gradually raise the temp. to 70° in 20–30 min. Maintain at 70° for 30 min., stirring the mixture from time to time, then increase the temp. to 80°, and keep it at that temp. for 10 min. Finally heat to the boiling point. Keep the mixtures well stirred. Cool the contents of the flasks and the water bath to 55°. Add 20 cc of the malt extract, mix well, and hold at 55° for 1 hour, stirring about once every 10 min. At the termination of the digestion rapidly increase the temp. to above 80°.

Measure out 316 cc of 95% alcohol. Add a portion, a little at a time, to the contents of the flask, with thoro shaking between additions. After cooling to room temp. adjust the volume with H<sub>2</sub>O so that the quantity of liquid is 500 cc, making allowance for the volume occupied by the charge by adding 3 cc of H<sub>2</sub>O for every 4 g of charge present after bringing the contents to the 500 cc mark. (The determination may be interrupted at this stage for several days. The volume should be re-adjusted if evaporation has occurred in the meantime.) Mix thoroly, breaking up any ropy coagulum as much as possible by pouring back and forth from one large beaker to another. Filter thru dry paper. Test the solid residue for starch, either microscopically or by the I color test, after elimination of alcohol and gelatinization with H<sub>2</sub>O. (If more than the merest trace of starch is found, reject the entire determination.) Evaporate exactly 200 cc of the filtrate on a steam bath to a volume of 15–20 cc, or until practically all alcohol has been expelled. Do not allow the evaporation to proceed to dryness.

Transfer the aqueous residue of starch conversion products to a 200 cc volumetric

flask with hot  $H_2O$ , using a rubber-tipped rod to recover any dextrin that may be present. Allow to cool somewhat, and complete the volume to 200 cc. Transfer the contents to a suitable digestion flask, add 20 cc of  $HCl$  (sp. gr. 1.125), made by diluting 68 cc of strong acid (sp. gr. 1.19, or 37%  $HCl$ ) to 100 cc, and connect the flask with a reflux condenser. Heat in a boiling water bath for 2.5 hours. Cool, and for samples of linseed meal or other material yielding solns which at this stage need further purification, add not more than 1 cc of a 10% soln of phosphotungstic acid in 1%  $HCl$ . Mix, and allow to stand at least 15 min. Increase the volume with  $H_2O$  to 250 cc in a volumetric flask, mix well, and filter thru dry paper. Partially neutralize 200 cc of the filtrate while stirring by adding 10 cc of a strong soln of caustic soda (44 g of  $NaOH$  per 100 cc of  $H_2O$ ) and nearly complete the neutralization with a little powdered anhydrous  $Na_2CO_3$ . Transfer to a 250 cc flask with  $H_2O$ , cool to room temp., make up to the mark, and thoroly mix. Filter, if necessary, and determine the dextrose in a 50 cc aliquot of the filtrate, gravimetrically, as directed under **XXXIV**, 46 or 48. Correct the weight of dextrose obtained by subtracting the weight of dextrose found for the same aliquot of the malt control, and multiply the corrected weight of dextrose by 0.90 to obtain the weight of starch.

$$\text{Aliquots: Charge} \times \frac{200}{500} \times \frac{200}{250} \times \frac{50}{250}, \text{ or Charge} \times 0.064.$$

#### PENTOSANS<sup>9</sup>—OFFICIAL

#### 35

##### REAGENTS

(a) *Hydrochloric acid*.—Contains 12% by weight  $HCl$ . To 1 volume of  $HCl$  add 2 volumes of  $H_2O$ . Determine the percentage of acid by titration against standard alkali and adjust to proper strength by dilution or addition of more strong acid, as may be necessary.

(b) *Phloroglucin*.—Dissolve a small quantity of phloroglucin in a few drops of acetic anhydride, heat almost to boiling, and add a few drops of  $H_2SO_4$ . A violet color indicates the presence of diresorcin. A phloroglucin which gives more than a faint coloration may be purified by the following method: Heat in a beaker about 300 cc of the dilute  $HCl$  and 11 g of commercial phloroglucin, added in small quantities at a time, stirring constantly until it is nearly dissolved. Pour the hot soln into a sufficient quantity of the same  $HCl$  (cold) to make the volume 1500 cc. Allow to stand at least overnight, preferably several days, to permit the diresorcin to crystallize. Filter immediately before using. A yellow tint does not interfere with its usefulness. In using, add the volume containing the required quantity of phloroglucin to the distillate.

#### 36

##### DETERMINATION

Place such a quantity of the sample, 2–5 g, that the weight of phloroglucide obtained shall not exceed 0.300 g, in a 300 cc distillation flask, together with 100 cc of the dilute  $HCl$  and several pieces of recently ignited pumice stone. Place the flask on a wire gauze, connect with a condenser, and heat, rather gently at first, and then regulating so as to distil over 30 cc in about 10 min. Pass the distillate thru a small filter paper. Replace the 30 cc distilled by a like quantity of the dilute acid, added by means of a separatory funnel in such a manner as to wash down the particles adhering to the sides of the flask, and continue the process until the distillate amounts to 360 cc. To the total distillate add gradually a quantity of phloroglucin dissolved in the dilute  $HCl$  and thoroly stir the resulting mixture. (The quantity

of phloroglucin used should be about double that of the furfural expected. The soln turns yellow, then green, and very soon there appears an amorphous greenish precipitate that grows darker rapidly, till it becomes almost black.) Make the soln up to 400 cc with the dilute HCl and allow to stand overnight.

Collect the amorphous black precipitate in a weighed Gooch crucible having an asbestos mat, wash carefully with 150 cc of H<sub>2</sub>O so that the H<sub>2</sub>O is not entirely removed from the crucible until the very last, and dry for 4 hours at the temp. of boiling H<sub>2</sub>O. Cool, and weigh in a weighing bottle. The increase in weight is taken to be furfural phloroglucide. To calculate the furfural, pentoses, or pentosans from the phloroglucide, use the following formulas given by Kröber:

(1) For a weight of phloroglucide, designated by "a" in the following formulas, under 0.03 g:

$$\text{Furfural} = (a + 0.0052) \times 0.5170.$$

$$\text{Pentoses} = (a + 0.0052) \times 1.0170.$$

$$\text{Pentosans} = (a + 0.0052) \times 0.8949.$$

In the above and also in the following formulas, the factor 0.0052 represents the weight of the phloroglucide that remains dissolved in the 400 cc of acid soln.

(2) For a weight of phloroglucide "a" between 0.03 and 0.300 g, use Kröber's table, XLII, 18, or the following formulas:<sup>11</sup>

$$\text{Furfural} = (a + 0.0052) \times 0.5185.$$

$$\text{Pentoses} = (a + 0.0052) \times 1.0075.$$

$$\text{Pentosans} = (a + 0.0052) \times 0.8866.$$

(3) For a weight of phloroglucide "a" over 0.300 g, use the following formulas:

$$\text{Furfural} = (a + 0.0052) \times 0.5180.$$

$$\text{Pentoses} = (a + 0.0052) \times 1.0026.$$

$$\text{Pentosans} = (a + 0.0052) \times 0.8824.$$

## 37

## GALACTAN TENTATIVE

Extract a convenient quantity of the sample, representing 2.5-3 g of the dry material, on a hardened filter with 5 successive portions of 10 cc of ether; place the extracted residue in a beaker, about 5.5 cm in diameter and 7 cm deep; add 60 cc of HNO<sub>3</sub> (sp. gr. 1.15); and evaporate on a steam bath to a volume of 20 cc. Let stand 24 hours, then add 10 cc of H<sub>2</sub>O, and allow to stand another 24 hours. Pass thru a filter and wash the impure mucic acid crystals with 30 cc of H<sub>2</sub>O to remove as much of the HNO<sub>3</sub> as possible, and return filter and contents to the original beaker. Add 30 cc of (NH<sub>4</sub>)<sub>2</sub>CO<sub>3</sub> soln (consisting of 1 part (NH<sub>4</sub>)<sub>2</sub>CO<sub>3</sub>, 19 parts H<sub>2</sub>O, and 1 part of NH<sub>4</sub>OH) and heat the mixture in a water bath, at 80°, for 15 min., with constant stirring. The (NH<sub>4</sub>)<sub>2</sub>CO<sub>3</sub> combines with the mucic acid, forming soluble NH<sub>4</sub> mucate. Wash the filter paper and contents several times with hot H<sub>2</sub>O by decantation, passing the washings thru a filter paper, to which finally transfer the residue, and wash thoroly. Evaporate the filtrate to dryness on a water bath, avoiding unnecessary heating which causes decomposition; add 5 cc of HNO<sub>3</sub> (sp. gr. 1.15); stir the mixture thoroly; and allow to stand for 30 min. Collect the precipitated mucic acid on a weighed Gooch crucible or other filter; wash with 10-15 cc of H<sub>2</sub>O, then with 60 cc of 95% alcohol, and then a number of times with ether; dry at the temp. of boiling H<sub>2</sub>O for 3 hours; and weigh. Multiply the weight of the mucic acid by 1.33 to convert to galactose, and by 1.20 to convert to galactan.

38

WATER-SOLUBLE ACIDITY<sup>12</sup>—TENTATIVE

Weigh 10 g of the sample into a shaking bottle, add 200 cc of  $H_2O$ , and shake for 15 min. Filter the extract thru a folded filter and take a 20 cc aliquot (equivalent to 1 g of sample). Dilute with 50 cc of  $H_2O$  and titrate with 0.1 N NaOH soln, using phenolphthalein indicator. Report results in terms of cc of 0.1 N NaOH required for neutralizing the extract from 1 g of material.

39

SALT<sup>13</sup> (QUALITATIVE) OFFICIAL

Transfer 2 cc of a 5% soln of  $AgNO_3$  to a small test tube of 1 cm internal diameter. Carefully add to this liquid an equal volume of the feed, which previously has been ground to pass a mm sieve, so that most of the sample floats or remains above the liquid. Gradually incline the tube so that the liquid is absorbed. White patches of  $AgCl$  appear wherever the minutest crystal of salt comes in contact with the soln. These patches may easily be observed with a lens or even with the naked eye.

40

RICE HULLS IN RICE BRAN<sup>14</sup>—TENTATIVE

Thoroughly mix the sample to be examined. Withdraw a small portion and grind until it passes thru a 60-mesh sieve. Weigh 4 mg on a slide ruled in parallel lines 1/20 in. apart or transfer to the ruled slide after weighing. Add just sufficient chloral hydrate soln (1+1) to fill in under the cover-glass, which, preferably, should be square (about 22 mm). After the cover-glass is in place, warm gently, but do not boil, to eliminate the starch masses and clear the tissues. Count the particles of hull tissue, using a microscope having a magnification of about 90 diameters. The high refraction and yellowish green color of the hull particles will aid in distinguishing the small pieces not easily recognized by their structure. (In order to avoid duplicate counting, it is well to disregard the particles that extend over the upper line of the strip.) Compare the results with those obtained on standards containing known quantities of hulls.

41

OAT HULLS IN OATS AND OAT FEEDS<sup>15</sup>—TENTATIVE

(Results are only approximate.)

Place in a 1000 cc beaker 800 cc of  $H_2O$  and 2 g of the sample, previously ground to pass thru a sieve having circular openings 1 mm in diameter. Stir vigorously to obtain a centrifugal effect, allow to stand for 5 min., and then decant the supernatant liquid carefully, retaining so far as possible all hull particles. Repeat this procedure several times until the supernatant liquid becomes clear, or nearly so, and then transfer the residue with the aid of 150 cc of  $H_2O$  to a 300 cc beaker. Add 5 drops of HCl and boil for 2 min., constantly stirring the mixture. Transfer to the original beaker with the aid of 500 cc of  $H_2O$ , stir, and allow to stand until the supernatant liquid is clear. Draw off the liquid by means of a siphon of rubber tubing having a 3 or 4 mm bore, using a pinch clamp to control the flow so that practically all the liquid may be siphoned off. (Tilting the beaker will also help to obtain this result.) If on standing a deposit forms, siphon again. Transfer the hulls with the aid of  $H_2O$  to a paper filter, wash several times with alcohol, and allow to dry to constant weight at room temp. When dry, carefully remove the hulls from the paper, using if necessary a small stiff brush, and weigh. (A weighed Gooch crucible may be used instead of the paper filter.) Multiply the weight of hulls by 50 to obtain the percentage of hulls in the sample.

**42 GRIT IN POULTRY AND SIMILAR FEEDS<sup>14</sup>—TENTATIVE**

Place 2 g of the prepared sample, 1, thoroly mixed, in an evaporating dish of about 30 cc capacity. Add about 5 cc of  $\text{CHCl}_3$  and mix gently with a glass rod so that the liquid comes in contact with all portions of the sample. Brush the particles adhering to the rod into the dish, and after pushing all particles down into the  $\text{CHCl}_3$  with a 25 mm circular or square cover-glass, use the glass to skim off or pull the floating portion of the material over the top of the dish, taking care not to submerge the cover-glass deep enough to disturb the grit settled at the bottom of the dish. After skimming until the surface of the  $\text{CHCl}_3$  is nearly clear, slowly pour the supernatant liquid into a second evaporating dish. Wash the sides of the dish with a few cc more of  $\text{CHCl}_3$  and repeat the skimming and decanting operation until no floating particles remain. (This will require 10–15 cc of  $\text{CHCl}_3$ .) When grit only remains, allow the last traces of  $\text{CHCl}_3$  to evaporate spontaneously, and weigh. Weight of residue  $\times 50$  = percentage of grit. After weighing examine the residue for impurities. Also pour out the  $\text{CHCl}_3$  washings collected in the second dish and observe whether any grit has been transferred to it during the process.

**43 BONE IN MEAT SCRAP OR TANKAGE<sup>15</sup>—TENTATIVE**

Separate the bone as directed under 42. In some instances it may be found necessary, after the first washing with  $\text{CHCl}_3$ , to rub the remaining residue of bone with a glass rod or small pestle in order to bring some of the adhering particles to the surface of the  $\text{CHCl}_3$ .

**44 CALCIUM OXIDE IN MINERAL FEEDS<sup>16</sup>—TENTATIVE**

Weigh a 2 g portion of the finely ground sample into a silica or porcelain dish and ignite in a muffle to a carbon-free ash, but avoid fusing. Boil the residue in 40 cc of  $\text{HCl}$  (1+3) and a few drops of  $\text{HNO}_3$ . Transfer to a 250 cc volumetric flask, cool, dilute to mark, and mix thoroly. Pipet 25 cc of the clear liquid into a beaker, dilute to about 100 cc, and add two drops of methyl red indicator. Add  $\text{NH}_4\text{OH}$  (1+1) dropwise to a pH of 5.6, as shown by the intermediate brownish color. If overstepped, add with a dropper  $\text{HCl}$  (1+3) to a brownish point. Add 2 drops  $\text{HCl}$  (1+3). The color should now be pink (pH 3.0–4.4) instead of brown. Dilute to about 150 cc, bring to boiling, and add slowly with constant stirring 20–30 cc of a saturated (4.2%) soln of  $(\text{NH}_4)_2\text{C}_2\text{O}_4$ , which should also be hot. If the red color changes to brown or yellow, add  $\text{HCl}$  (1+3) dropwise until the color again changes to pink. Let stand overnight to allow precipitate to settle. Filter the supernatant liquid thru quantitative filter paper on a Gooch crucible, or on a fritted glass filter (Jena 1G4 is preferable), and wash the precipitate thoroly with  $\text{NH}_4\text{OH}$  (1+50). Place the filter paper or crucible with the precipitate in the original beaker, and add a mixture of 125 cc of  $\text{H}_2\text{O}$  and 5 cc of  $\text{H}_2\text{SO}_4$ . Heat to 70° or above and titrate with 0.1 N  $\text{KMnO}_4$  until the first slightly pink color is obtained. Presence of filter paper may cause the pink color to fade in a few seconds. Correct for the blank and calculate the percentage of  $\text{CaO}$  in the sample.

**CYANOGENETIC GLUCOSIDES IN FEEDS AND SIMILAR MATERIALS<sup>17</sup>****45***Qualitative Test*

Prepare sodium picrate paper by dipping strips into a 1% soln of picric acid and drying, then dipping into a 10% soln of  $\text{Na}_2\text{CO}_3$  and drying. Preserve these papers in a stoppered bottle. Finely chop a small quantity of plant material and place in



a test tube. Insert a piece of the moist sodium picrate paper in the tube, taking care that it does not come in contact with the material. Add a few drops of  $\text{CHCl}_3$  and stopper the tube tightly. The sodium picrate paper gradually turns orange, then brick red if the plant tissue contains cyanogenetic glucosides. (The test is delicate, and the rapidity of the change in color depends upon the amount of free hydrocyanic acid present. This test works well with fresh plant materials, but in the case of relatively dry substances, particularly the seeds of various plants, the material should be ground and moistened with  $\text{H}_2\text{O}$  and allowed to hydrolyze in a stoppered test tube containing sodium picrate paper. If necessary, a small amount of emulsin may be added.)

#### HYDROCYANIC ACID FORMED BY THE HYDROLYSIS OF GLUCOSIDES IN BEANS<sup>43</sup>

46

##### *Acid Titration Method—Tentative*

Grind the sample to pass a 20-mesh sieve. Introduce 10–20 g of the ground material into an 800 cc Kjeldahl flask, add 100 cc of  $\text{H}_2\text{O}$ , and macerate at room temp. for 2 hours. Add 100 cc of  $\text{H}_2\text{O}$  and distil with steam, collecting the distillate in 20 cc of 0.02  $N$   $\text{AgNO}_3$  soln acidified with 1 cc of  $\text{HNO}_3$ . Before distilling, adjust the apparatus so that the tip of the condenser dips below the surface of the liquid in the receiver. When 150 cc has passed over, filter the distillate thru a Gooch crucible; wash the receiver and Gooch with a little  $\text{H}_2\text{O}$ ; and titrate the excess of  $\text{AgNO}_3$  in the combined filtrate and washings with 0.02  $N$   $\text{KCNS}$  soln, using ferric alum indicator. 1 cc of 0.02  $N$   $\text{AgNO}_3$  soln = 0.54 mg of  $\text{HCN}$ .

47

##### *Alkaline Titration Method—Tentative*

Place 10–20 g of the sample, ground to pass a 20-mesh sieve, into an 800 cc Kjeldahl flask, and add about 200 cc of  $\text{H}_2\text{O}$ . (The autolysis should be conducted with the apparatus completely connected for distillation.) Distil with steam and collect 150–160 cc of distillate in a soln of  $\text{NaOH}$  (0.5 g in 20 cc of  $\text{H}_2\text{O}$ ). It is preferable to dilute to a volume of 250 cc and titrate a 100 cc aliquot.

To 100 cc of the distillate add 8 cc of 6  $N$   $\text{NH}_4\text{OH}$  and 2 cc of a 5% soln of  $\text{KI}$  and titrate with 0.02  $N$   $\text{AgNO}_3$ , using a micro buret. The end point is a faint but permanent turbidity, which may be easily recognized, especially against a black background. 1 cc of 0.02  $N$   $\text{AgNO}_3$  = 1.08 mg of  $\text{HCN}$ .

48

##### *Prussian Blue Method—Tentative*

Macerate and distil 10–20 g of the sample as directed under 46, using in the receiver a soln containing 0.5 g of  $\text{NaOH}$  dissolved in 20 cc of  $\text{H}_2\text{O}$ , and dilute the distillate to 200 cc in a volumetric flask. Concentrate 20 cc of this soln, which must contain a slight excess of  $\text{NaOH}$ , in a 200 cc round-bottomed flask attached to a vacuum pump and condenser, heating the flask in a water bath below  $70^\circ$ . (An adapter may be used to avoid loss by spattering.) When the volume has been reduced to 1 cc or less, add 0.2–0.5 cc of freshly prepared 3%  $\text{FeSO}_4$  soln and about 0.5 g of  $\text{KF}$ . Exhaust the flask at once by means of a vacuum pump. Mix the contents by rotating the flask. After 5–10 min. detach the flask and acidify the mixture with 30%  $\text{HNO}_3$ . The blue color usually appears at once, altho in case traces only are present it is sometimes necessary to warm to about  $50^\circ$  in a water bath. Dilute the resulting suspension of Prussian blue to a convenient volume and compare the color with a standard Prussian blue mixture, prepared as directed above from the

vacuum evaporation of a standard soln containing 1 mg of KCN diluted to 25 cc. This soln, containing 1 mg of KCN, = 0.415 mg of HCN.

49

**FERROUS SULFATE<sup>a</sup>—TENTATIVE**

Sift a portion of the feed thru a fine sieve (40-mesh) over a sheet of white glazed paper whose entire surface has been moistened with a soln of potassium ferricyanide (1+10) in such a manner that the feed will be distributed thinly over the area of the paper. After a few moments wash off the feed under a slow stream of H<sub>2</sub>O. A blue speck or spot denotes a particle of ferrous salt.

50

**COPPER SULFATE<sup>a</sup>—TENTATIVE**

Proceed as directed under 49, except to use a soln of ferrocyanide (1+10). A brown speck or spot denotes a particle of copper salt.

51

**POTASSIUM IODIDE<sup>a</sup>—TENTATIVE**

Sift a portion of the feed over a sheet of white glazed paper whose entire surface has been moistened with a mixture of starch indicator and Br water (3 parts of the former to 1 of the latter) in such a manner that the feed will be distributed thinly over the area of the paper. A blue coloration denotes a particle of an iodide. If an extremely small quantity of KI is to be detected, modify the above procedure by carefully charring 10 g or more of the feed, washing the residue with a small amount of H<sub>2</sub>O, and evaporating the filtered soln in a white evaporating dish so that the solids are concentrated on one small spot.

When moistened with the starch indicator and Br water a blue coloration denotes the presence of an iodide.

**IODINE IN MINERAL MIXED FEEDS***Knapheide-Lamb Method<sup>20</sup>—Tentative*

52

**REAGENTS**

(a) *Reduced phosphoric acid*.—20%. Reduce impurities in the H<sub>3</sub>PO<sub>4</sub> according to Kendall's method<sup>21</sup> by diluting the 85% acid with 4 volumes of H<sub>2</sub>O and boiling for some time with Al strips.

(b) *Sodium thiosulfate soln*.—0.005 N. Preferably standardize by pipetting into a beaker 25 cc of a soln containing 0.1308 g of KI per liter and adding 200 cc of H<sub>2</sub>O, 5 cc of 20% NaHSO<sub>3</sub> soln, and 2 or 3 g of NaOH. Neutralize the mixture with sirupy H<sub>2</sub>PO<sub>4</sub>, adding 1.0 cc in excess and proceeding as directed in the regular determination. To calculate the mg of I to which 1 cc of the Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> soln is equivalent,

use the following formula: 
$$\frac{2.5}{\text{cc of Na}_2\text{S}_2\text{O}_3 \text{ soln}}$$
 (It is well to standardize the Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>

soln the day the determination is made.)

53

**APPARATUS<sup>22</sup>**

*Furnace*.—Use a sheet-iron cylinder 4 in. in dia. and 12 in. high, and have an opening in the center of the top large enough to accommodate a 100 cc nickel crucible. Suspend a 2½ in. circular plate in the center of the cylinder 3 in. below the top, for spreading the flame, thereby preventing the free flame from coming in contact with the crucible, and providing uniform heat. Make a slot at the bottom of the cylinder 1 in. wide by 3 in. high for admitting air and the burner tubing, and near the top rim make eight ¼ in. holes to allow for the escape of the exhaust gases.

Fuse together in a 100 cc nickel crucible 20 g of NaOH and 10 g of KNO<sub>3</sub> and cool. Place evenly on top of the fused alkali a 1 to 10 g sample (depending upon its composition and the trouble experienced from frothing in the fusion) of the mineral mixture and completely moisten with 5 cc of saturated NaOH soln and 10 cc of 80% alcohol. Place the crucible on a cold three-heat hot plate and evaporate the alcohol by the low heat. After 30 min. cautiously increase the heat until the crucible has been subjected to the highest temp. of the hot plate for 1½–2 hours. (Thoro heating at this time prevents most of the trouble from effervescence of the material during the fusion.) Then place the crucible in the furnace described above or in a similar furnace.

To prevent loss give close attention during the fusion to mineral mixtures containing charcoal or organic matter because of the violent reaction between the C and the KNO<sub>3</sub>. If the reaction becomes too violent, lift the crucible from the furnace for a moment, and if necessary cool the bottom of the crucible in a beaker of H<sub>2</sub>O. When the mixture is in a quiet state of fusion tip the crucible on all sides in an open flame to wash down the fusion mixture. Add a few small crystals of KNO<sub>3</sub> until no more gas is liberated by further additions, and again wash down the sides of the crucible in the flame.

Pour the melt out into the clean crucible cover to cool, or turn the crucible while cooling so that the material solidifies on the sides. Place the cooled melt and the crucible in a 600 cc beaker, cover with H<sub>2</sub>O, and heat below the boiling point for a short time. After allowing the mixture to stand overnight at room temp., rinse off the crucible and cover and remove. In order to neutralize part of the alkali and facilitate filtering, add 10 cc of sirupy H<sub>3</sub>PO<sub>4</sub> and place the beaker on a steam bath for 3–4 hours, stirring occasionally to break up the mass and insure complete solution of the I. Cool the beaker, filter off the insoluble residue into a 10 cm funnel and wash with cold H<sub>2</sub>O into an 800 cc beaker, adjusting the volume to 550–600 cc. (The soln should be clear and colorless.)

In order to destroy nitrites, which interfere with the titration with methyl orange, add 10 cc of 20% NaHSO<sub>3</sub>, bring the soln just to the boiling point, and cool. Run approximately 30 cc of 85% H<sub>3</sub>PO<sub>4</sub> in from a buret, add a few drops of methyl orange soln, continue the addition of H<sub>3</sub>PO<sub>4</sub> to the neutral color of the methyl orange, and finally add 1.5 cc of H<sub>3</sub>PO<sub>4</sub> in excess. (The total quantity of H<sub>3</sub>PO<sub>4</sub> required is generally not over 35 cc, except when the presence of considerable C in the sample has necessitated the use of more KNO<sub>3</sub>, which is thus mainly reduced to carbonate.) Use care not to run appreciably over the end-point, as excess acid gives low results. However, the addition of the acid must be fairly rapid, as the color of the methyl orange has a tendency to fade, due to incomplete destruction of the nitrites.

After neutralization, add a small lump of anthracite coal (0.5 cm in diameter) and boil the soln for at least 20 min., the volume being reduced to about 400–500 cc. (Boiling is essential to remove all traces of sulfurous acid.) Again cool the soln and add Br water until a distinct and permanent yellow color is produced. Boil the soln until colorless by reflected light and then for exactly 5 min. longer. Add a few crystals of salicylic acid to assure the removal of the last traces of Br, cool the soln, and add 5 cc of 20% reduced H<sub>3</sub>PO<sub>4</sub> and 0.5–1.0 g of C.P. KI. Titrate the soln in the usual manner with 0.005 N Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>, adding starch soln when the brown color of the liberated I is nearly gone. (The volume of the soln at the final titration should be 400 to 500 cc.)

VITAMIN D ASSAY BY PREVENTIVE BIOLOGICAL TEST<sup>2</sup>

(Applicable to fish and fish liver oils and their extracts, and to materials used for supplementing the vitamin D content of feeds. Not applicable to irradiated ergosterol products or to irradiated yeast unless recommended for poultry.)

55

## BASAL RACHITIC RATION

	<i>per cent</i>
Ground yellow corn.....	59
Pure wheat flour middlings.....	25
Crude domestic acid-precipitated casein.....	12
Calcium carbonate (precipitated).....	1
Calcium phosphate (precipitated).....	1
Iodized salt (.02% KI).....	1
Non-irradiated yeast (7% minimum N).....	1

56

## PROCEDURE

Place groups of 10 or more 1-day-old white leghorn chickens in screen-bottomed biological cages or a battery brooder out of direct sunlight (Red electric light bulbs are satisfactory as a source of heat for the cages.) Reserve one group for negative control purposes, and one or more additional groups for each material to be assayed. Keep distilled H<sub>2</sub>O before the chicks at all times.

Prepare sufficient basal rachitic ration for the entire feeding period (80 lbs. per 100 birds is ample). Prepare the supplemented rations at 8-12 day periods. Supplement the basal rachitic ration with corn oil in a quantity equal to the maximum addition of the oil to be assayed. (This is the ration to be fed to the negative control group.) Supplement the basal ration with different levels of the material to be assayed. Add corn oil to bring the percentage of oil up to that added to the negative control ration. (These are the rations to be fed to the other groups.)

On the second day give the groups two 15 min. feedings of their respective rations. Beginning the third day feed the rations *ad libitum* for 28 days.

Kill the birds, remove the left tibia of each bird, and clean of adhering tissue. (To facilitate removal of adhering tissue the bones may be placed in boiling H<sub>2</sub>O for not more than 2 min.) Number the bones and place in 95% ethyl alcohol. Crush, wrap individually in filter paper, and extract the bones for 20 hours with hot 95% ethyl alcohol, followed by 20 hours with ethyl ether. (Other solvents may be used for this fat extraction.) Dry in a moisture oven, and store in a desiccator. Determine the percentage of ash of the moisture and fat-free bones by igniting in a muffle furnace at approximately 850° for 1 hour. Compile group ash averages.

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- <sup>6</sup> *Ibid.*, **7**, 339 (1924).
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- <sup>16</sup> Ibid., **5**, 424 (1922).
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## XXVIII. MEAT AND MEAT PRODUCTS

### MEAT

1

#### PREPARATION OF SAMPLE—OFFICIAL

To prevent loss of  $H_2O$  during preparation and subsequent handling do not use small samples. Keep the ground material in glass or similar containers provided with air- and water-tight covers. Prepare samples for analysis in the following manner:

(a) *Fresh meats, dried meats, cured meats, smoked meats, etc.*—Separate as completely as possible from any bone; pass rapidly thru a food chopper 3 times, thoroly mixing after each grinding; and begin all determinations as soon as practicable. If any delay occurs, chill the sample to inhibit decomposition.

(b) *Canned meats.*—Pass the entire contents of a can thru a food chopper, as directed under (a).

(c) *Sausages.*—Remove from casings and pass thru a food chopper, as directed under (a).

Dry the portions of the samples under (a), (b), and (c) not needed for immediate analysis, either in vacuo below  $60^\circ$  or by evaporating on a steam bath 2 or 3 times with alcohol. Extract the fat from the dried product with gasoline (b.p. below  $60^\circ$ ) and allow the gasoline to evaporate spontaneously, finally expelling the last traces by heating for a short time on a steam bath. Do not heat the sample or the separated fat longer than necessary because of tendency to decompose. Reserve the fat for examination as directed under XXXI, keeping it in a cool place, and complete the examination before it becomes rancid.

2

#### MOISTURE—OFFICIAL

Proceed as directed under XXVII, 2 or 6, following 6 when the dried sample is to be used for further determinations.

3

#### ADDED WATER IN SAUSAGE AND SIMILAR MEAT PRODUCTS—TENTATIVE

(a) *Moisture.*—Weigh accurately about 10 g of the ground sample into a tared weighing bottle, approximately 2 in. in diameter, containing a short glass rod flattened at one end. Remove 2.5 g for the protein determination. Reweigh the remainder in the bottle, spreading it out in a thin layer over the sides and bottom by means of the glass rod, and use this sample for the determination of moisture. Dry in air at atmospheric pressure at a temp. of  $101$ – $102^\circ$  for approximately 16–18 hours, or at a temp. of approximately  $125^\circ$  (not lower than  $120^\circ$  nor higher than  $130^\circ$ ) for approximately 2–3 hours, or until no significant loss of weight occurs on subsequent drying for a period of 1–2 hours. If preferred, determine moisture as directed under 2.

(b) *Nitrogen.*—Determine total N as directed under II, 19, 23, or 25. Protein = total N  $\times 6.25$ .

(c) *Added water.*—Multiply the percentage of protein calculated from the N determination by 4 and subtract the result from the percentage of moisture found. Report the difference, if any, as added  $H_2O$ .

4

#### ASH—OFFICIAL

Proceed as directed under XXVII, 8.

5

**SALT<sup>2</sup>**

Moisten 2½–3 g of the finely comminuted and thoroly mixed sample in a platinum dish with 20 cc of 5% sodium carbonate soln, evaporate to dryness, and ignite at a temp. not exceeding dull redness. Extract with hot H<sub>2</sub>O, filter, and wash. Return the residue to the platinum dish and ignite to an ash. Dissolve the ash in HNO<sub>3</sub> (1+4), filter to free from any insoluble residue, wash thoroly, and add the wash soln to the H<sub>2</sub>O extract. Determine Cl in the combined filtrate and washings as directed in XII, 37.

6

**CRUDE FAT OR ETHER EXTRACT—OFFICIAL**

Proceed as directed under XXVII, 22.

7

**TOTAL PHOSPHORUS—OFFICIAL**

Destroy the organic matter as directed under II, 8(c) or (d), and proceed as directed under II, 9 or 12.

8

**TOTAL NITROGEN<sup>1</sup>—OFFICIAL**

Proceed as directed under II, 21, 23, or 25, using about 2 g of the fresh sample.

**AMMONIA***Aeration Method<sup>4</sup>—Tentative*

9

**APPARATUS**

Use the apparatus illustrated in Fig. 30. *A* is a wash bottle ½ full of H<sub>2</sub>SO<sub>4</sub> (1+9); *B* is a tube containing the sample; *C* is a rubber disk; and *D* is a 5 cc bulb to prevent spray from being carried over into the tube *E*, which contains the standard acid; *F* is a safety bottle.

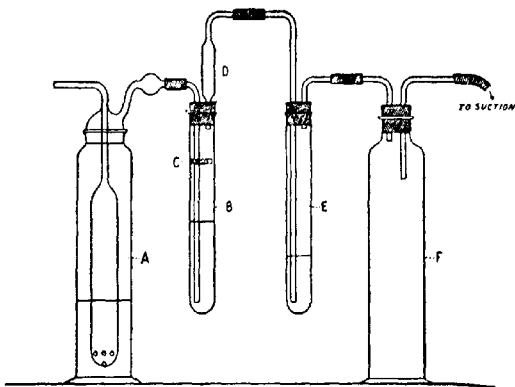


FIG. 30.—APPARATUS FOR THE DETERMINATION OF AMMONIA

10

**DETERMINATION**

Introduce 2–4 g of the finely divided meat into tube *B* and add 20 cc of NH<sub>3</sub>-free H<sub>2</sub>O. Place a measured quantity of 0.04 *N* or 0.02 *N* H<sub>2</sub>SO<sub>4</sub> or HCl in tube *E*. Add

1 cc of saturated K oxalate soln to the sample in tube *B*, introduce a few drops of kerosene, and finally add just sufficient saturated Na or K carbonate soln to render the mixture alkaline. Place the tubes in position at once, pass air thru the apparatus, and titrate the standard acid in tube *E* at hourly intervals until  $\text{NH}_3$  ceases to be given off, using methyl red, cochineal, or congo red indicator. (If preferred, the  $\text{NH}_3$  collected in tube *E* may be determined by nesslerizing as directed under XXXVII, 11.)

#### NITRATES (INCLUDING ALSO NITRITES)

##### *Ferrous Chloride Method<sup>b</sup>—Tentative*

#### 11

##### REAGENTS

(a) *Ferrous chloride soln.*—Dissolve 400 g of nails, tacks, or other small pieces of iron in a 2 liter Florence flask with 1 liter of HCl, excluding the air from the flask by means of a stopper equipped with a Bunsen valve. When the evolution of gas ceases, transfer, and keep the soln in completely filled 50 cc glass-stoppered bottles. Use only freshly opened bottles of the reagent for the determination.

(b) *Standard sodium nitrate soln.*—Dissolve 2 g of  $\text{NaNO}_3$  in 1 liter of recently boiled  $\text{H}_2\text{O}$ . Determine NO in 50 cc of this soln (equivalent to 0.1 g of  $\text{NaNO}_3$ ) as directed under 13.

#### 12

##### APPARATUS

Clamp a 500 cc Kjeldahl flask fitted with a 2-holed stopper to an iron stand. Thru one of the holes pass the stem of a 100–125 cc cylindrical separatory funnel having a glass stopcock, and into the other fit a delivery tube leading downward at an angle from the flask into a trough containing a soln of commercial NaOH (1+1). Terminate the upper end of the delivery tube just below the stopper in the flask and place the lower end, which is slightly constricted, bent upward, and covered with rubber tubing to prevent fracture, under the surface of the NaOH soln in the trough, the exit being just below the mouth of an inverted measuring tube (50 cc plain eudiometer tube) filled with the soln of NaOH. A single coil of tin tubing fitted into the trough and carrying a current of cold  $\text{H}_2\text{O}$  greatly facilitates the determination.

#### 13

##### DETERMINATION

Extract 100 g of the sample by boiling 6–7 times with successive 35–50 cc portions of  $\text{H}_2\text{O}$ , decant the extracts thru a muslin or paper filter into a casserole, and evaporate the combined extracts to a volume of about 50 cc. Introduce 50 cc of the  $\text{FeCl}_2$  soln and 50 cc of HCl (1+2.5) into the Kjeldahl flask, close the stopcock of the funnel, move the end of the delivery tube so that the escaping air will not pass into the measuring tube, and boil the contents of the flask until the air is completely expelled. Place the exit end of the delivery tube beneath the measuring tube and boil the contents of the flask 1 min. longer to make certain that no air remains. Introduce the concentrated extract of the sample into the flask, a little at a time thru the funnel, continuously boiling the contents of the flask to force the NO gas into the measuring tube. Finally rinse the casserole and the funnel 3 or 4 times with 5–10 cc of recently boiled  $\text{H}_2\text{O}$ , adding the rinsings to the contents of the evolution flask in the manner described above. When the evolution of gas ceases, cover the opening of the measuring tube with a porcelain crucible, using tongs, and carefully transfer the tube to a tall glass jar containing a soln of NaOH (1+1), kept at room temp. The temp. of the surrounding caustic soln will soon (10–15 min.) be imparted



to the contents of the tube, and the volume of NO is read with the tube in such a position that the level of the solns within the tube coincides with the level outside. Calculate the percentage of nitrates and nitrites as  $\text{NaNO}_2$  from the volume of NO obtained from the sample compared with the volume obtained from 0.1 g of  $\text{NaNO}_2$ , both measured under identical conditions.

After the measuring tube has been removed, quickly insert another filled with a strong soln of commercial NaOH (1+1) over the delivery tube and boil 1 min. longer to make sure that all the NO has been expelled. Run another 50 cc portion of the standard soln into the apparatus and repeat the determination. Then run the samples in the same manner, in each case making certain that all the NO gas has been expelled. After 6 to 8 determinations have been made, excluding the 2 standards, finally run another standard. The 3 standards should check within 0.5 cc on about 30–35 cc; 0.1 g of  $\text{NaNO}_2$  should give 26.36 cc of NO at 0° and 760 mm pressure. Report results as percentage of  $\text{NaNO}_2$ .

*Phenoldisulfonic Acid Method<sup>8</sup>—Tentative*

## 14

## REAGENTS

(a) *Phenoldisulfonic acid soln.*—Heat 6 g of phenol with 37 cc of  $\text{H}_2\text{SO}_4$  on a steam bath, cool, and add 3 cc of  $\text{H}_2\text{O}$ .

(b) *Standard comparison soln.*—Dissolve 1 g of pure, dry  $\text{NaNO}_2$  in  $\text{H}_2\text{O}$  and dilute to 1 liter. Evaporate 10 cc of this soln to dryness on a steam bath, add 2 cc of the phenoldisulfonic acid soln, mix quickly and thoroly by means of a glass rod, heat for about a minute on a steam bath, and dilute to 100 cc. 1 cc of the diluted soln = 0.1 mg of  $\text{NaNO}_2$ . Prepare a series of standard comparison tubes by introducing quantities ranging from 1 to 20 cc of the diluted soln (0.1–2.0 mg of  $\text{NaNO}_2$ ) into 50 cc Nessler tubes, adding 5 cc of  $\text{NH}_4\text{OH}$  to each and diluting to 50 cc. The standard tubes thus prepared are permanent for several weeks if kept tightly stoppered.

## 15

## DETERMINATION

Weigh 1 g of the sample into a 100 cc flask, add 20–30 cc of  $\text{H}_2\text{O}$ , and heat on a steam bath for 15 min., shaking occasionally. Add 3 cc of a saturated nitrate-free  $(\text{Ag})_2\text{SO}_4$  soln for each per cent of NaCl present, then 10 cc of basic Pb acetate soln and 5 cc of alumina cream, shaking after each addition. Make up to the mark with  $\text{H}_2\text{O}$ , shake, and filter thru a folded filter, returning the filtrate to the filter until it runs thru clear. Evaporate 25 cc of the filtrate to dryness, add 1 cc of the phenoldisulfonic acid soln, mix quickly and thoroly by means of a glass rod, add 1 cc of  $\text{H}_2\text{O}$  and 3 or 4 drops of  $\text{H}_2\text{SO}_4$ , and heat on a steam bath for 2–3 min., being careful not to char the material. Then add about 25 cc of  $\text{H}_2\text{O}$  and an excess of  $\text{NH}_4\text{OH}$ , transfer to a 100 cc volumetric flask, add 1–2 cc of alumina cream if not perfectly clear, dilute to volume with  $\text{H}_2\text{O}$ , and filter. Fill a 50 cc Nessler tube to the mark with the filtrate and determine the quantity of  $\text{NaNO}_2$  present in the sample by comparison with the standard comparison tubes. If the soln is too dark for comparison with the standards, dilute with  $\text{H}_2\text{O}$ , and correct the result accordingly. Report as percentage of  $\text{NaNO}_2$ .

## 16

NITRITES<sup>2</sup>—TENTATIVE

(Applicable to cured meats.)

Weigh 5 g of the finely comminuted and thoroly mixed sample into a 50 cc beaker. Add approximately 40 cc of nitrite-free  $\text{H}_2\text{O}$  heated to a temp. of 80°. Mix thoroly

by stirring with a glass rod, taking care to break up all lumps, and transfer to a 500 cc graduated flask. Wash out the beaker and rod thoroly with successive portions of the hot  $\text{H}_2\text{O}$ , adding all washings to the flask. Add sufficient hot  $\text{H}_2\text{O}$  to bring the contents of the flask to a volume of approximately 300 cc, transfer the flask to the steam bath, and let stand for 2 hours, shaking occasionally. Add 5 cc of saturated  $\text{HgCl}_2$  soln and mix. Cool to room temp., make up to the mark with nitrite-free  $\text{H}_2\text{O}$ , and mix again. Filter, and determine nitrite N in a suitable aliquot as directed under XXXVII, 15, reporting results as part of  $\text{NaNO}_2$  per million.

#### STARCH

(In chopped meat, sausage, deviled meat, etc.)

17

##### *Qualitative Test—Tentative*

Treat 5-6 g of the sample with boiling  $\text{H}_2\text{O}$  for 2-3 min., cool the mixture, and test the supernatant liquid with I soln, XXXIII, 28(f). (In interpreting this test it should be remembered that a small quantity of starch may be present as the result of the use of spices. If a marked reaction is given, however, it may be concluded that starch or flour has been added, and a quantitative determination should be made. The qualitative test may be replaced by a microscopic examination, which discloses not only the presence of added starch but also the variety used.)

18

##### *Quantitative Method<sup>a</sup>—Tentative*

Treat in a 200 cc beaker 10 g of the finely divided sample with 75 cc of an 8% soln of KOH in 95% alcohol and heat on a steam bath until all the meat is dissolved (30-45 min.). Add an equal volume of 95% alcohol, cool, and allow to stand for at least an hour. Filter by suction thru a thin layer of asbestos in a Gooch crucible. Wash twice with a warm 4% soln of KOH in alcohol, 50% by volume, and then twice with warm 50% alcohol. Discard the washings. Retain as much of the precipitate in the beaker as possible until the last washing. Place the crucible with contents in the original beaker and add 40 cc of  $\text{H}_2\text{O}$  and 25 cc of  $\text{H}_2\text{SO}_4$ . Stir during the addition of the acid and make sure that the acid comes in contact with all the precipitate. Allow to stand about 5 min., add 40 cc of  $\text{H}_2\text{O}$ , and heat just to boiling, stirring constantly. Transfer the soln to a 250 cc volumetric flask, add 2 cc of 20% phosphotungstic acid soln, allow to cool to room temp., and make up to the mark with  $\text{H}_2\text{O}$ . Filter thru a starch-free filter paper, pipet 100 cc of the filtrate into a 200 cc volumetric flask, neutralize with 10% NaOH soln, make up to volume, and determine the dextrose present in a 50 cc portion of the filtrate as directed under XXXIV, 37, titrating the  $\text{Cu}_2\text{O}$  precipitate as directed under XXXIV, 40. Weight of dextrose  $\times 0.9$  = weight of starch.

#### GLYCOGEN

19

##### *Qualitative Test<sup>b</sup>—Tentative*

Boil 50 g of the macerated sample with 50 cc of  $\text{H}_2\text{O}$  for 15-30 min. Filter the broth thru moistened filter paper or fine linen. To a portion of the filtrate in a test tube add a few drops of a mixture of 2 parts of I, 4 parts of KI, and 100 parts of  $\text{H}_2\text{O}$ . If a considerable quantity of glycogen is present, it produces a dark brown color; this color is destroyed by heating, but it reappears on cooling. If starch is present, it may be precipitated by treating the water extract with two volumes of glacial acetic acid and after filtering applying the test for glycogen to the filtrate.

*Quantitative Method<sup>10</sup>—Tentative*

20

## PREPARATION OF SOLUTION

Weigh by difference about 25 g of the finely ground and thoroly mixed sample. Place in a 400 cc beaker and mix with 50 cc of KOH soln (1.5+1), free from carbonate. Cover the beaker with a watch-glass and digest on a steam bath for 2 hours, stirring occasionally. At the end of the 2 hours, dilute to approximately 200 cc with cold  $H_2O$ .

21

## DETERMINATION

Add to the soln, 20, an equal volume of 95% alcohol, cover with a watch-glass, and set aside for 10–12 hours. Decant the supernatant liquid thru a folded 18.5 cm filter, allowing the glycogen to remain in the beaker, and wash by decantation with 66% alcohol (2 volumes of 95% alcohol+1 of  $H_2O$ ) until the glycogen is white, or nearly so. (Usually about 4 washings are required.) Transfer the washed precipitate from the beaker to the filter and wash 2 or 3 times with the 66% alcohol. (The soln filters slowly, and the funnel should be covered with a watch-glass to prevent excessive evaporation. The albuminous substance present retards the filtration if it is permitted to dry on the paper. If the washing by decantation is not made as complete as possible, it will be difficult to obtain the glycogen free from the coloring matter.)

After the washing is completed, close the bottom of the funnel by a piece of rubber tubing and a pinch-cock. Fill the funnel with warm  $H_2O$ , cover with the watch-glass, and let stand 2–3 hours, or overnight. Open the pinch-cock and allow all the soln to pass thru the filter into a beaker. Close the funnel with the pinch-cock and fill with warm  $H_2O$  as before. Allow this  $H_2O$  to remain in the funnel for 1 hour and then filter as before. At first the glycogen soln appears quite turbid. Continue washing with warm  $H_2O$  until the filtrate becomes perfectly clear. To the soln of glycogen in  $H_2O$ , add double its volume of 95% alcohol and let stand overnight to complete the reprecipitation of the glycogen. Filter, and wash as before with 66% alcohol.

If desired, the last filtration may be made thru a weighed Gooch crucible and the weight of glycogen may be determined after drying to constant weight. This gives results that are approximately correct. More satisfactory results are obtained by hydrolyzing the glycogen with HCl (1+3) and determining the resultant dextrose. Dissolve the glycogen on the filter in warm  $H_2O$  as directed above, collecting the filtrate and washings in a 300 cc volumetric flask and keeping the volume within 225 cc. Add 12.5 cc of HCl to the combined filtrate and washings, mix, and place in a boiling water bath for 3 hours. Cool, neutralize with 10% NaOH soln, cool again, make up to volume with  $H_2O$ , and determine dextrose in an aliquot of the soln as directed under XXXIV, 37, determining the reduced Cu as directed under XXXIV, 40. The corresponding weight of dextrose  $\times 0.9$  = its equivalent of glycogen. Correct this result for dilution to obtain the percentage of glycogen in the sample.

22

## SUGAR—TENTATIVE

Weigh 100 g of the finely ground sample into a 600 cc beaker, add 200 cc of  $H_2O$ , heat to boiling, and boil gently for 5 min. Stir the contents of the beaker frequently during this and subsequent extractions to prevent bumping. (When several samples are extracted at the same time a mechanical stirring device is practically a necessity.) Remove the beaker from the flame, allow the insoluble matter to settle, and decant the clear liquid on an asbestos mat in a 4-inch funnel. Filter with the aid of

suction. Add 150 cc of hot  $H_2O$  to the residue in the beaker, boil gently for 5 min., let settle, and decant the clear liquid as directed previously. Repeat the operation, finally transfer the contents of the beaker to the funnel, wash with 150-200 cc of hot  $H_2O$ , and press the meat residue as dry as possible. Transfer the contents of the filter flask to an evaporating dish and evaporate on a steam bath to a volume of about 25 cc but not to dryness. Transfer the extract to a 100 cc volumetric flask, taking care that the volume of liquid does not exceed 60 cc. Add 25-35 cc of phosphotungstic acid soln (1+1), shake vigorously, let stand a few min. for gas bubbles to rise to the surface, make to volume, shake, and either filter or centrifuge. (The use of a centrifuge is to be preferred, because a larger volume of liquid is obtained.) Test a portion of the filtrate with dry phosphotungstic acid for complete precipitation. If an appreciable precipitate forms, take an aliquot of the filtrate, add 5-10 cc of the phosphotungstic acid soln, make to volume, filter, and test the filtrate for complete precipitation. The filtrate should also show not more than a slight reaction for creatinin when tested by adding to 5 cc a few drops of a saturated aqueous soln of picric acid and making the mixture alkaline with a few drops of 10% NaOH soln.<sup>11</sup>

Transfer 50 cc of the clarified extract to a 100 cc volumetric flask, add 5 cc of HCl, and invert the soln as directed under **XXVII**, 31. Cool the soln, neutralize to litmus, cool, make to volume, and filter. To the filtrate add sufficient dry powdered KCl to precipitate the excess of phosphotungstic acid, filter, test the filtrate for complete precipitation, and determine the reducing sugar as directed under **XXXIV**, 33 or 37, ascertaining the quantity of reduced Cu as directed under **XXXIV**, 41. Calculate the total sugar as dextrose.

If an abnormal reduction is obtained when the clarified meat extract is boiled with Fehling's soln, i.e., if the soln turns yellow, brown, green, or muddy in appearance instead of reddish-blue, discard the determination, since incomplete precipitation of the nitrogenous compounds, due to the use of insufficient phosphotungstic acid, is indicated.

**23****PRESERVATIVES—OFFICIAL**

Proceed as directed under **XXXII**.

**24****METALS—TENTATIVE**

Proceed as directed under **XXIX**.

**25****COLORING MATTERS—TENTATIVE**

Proceed as directed under **XXI**.

**SOLUBLE AND INSOLUBLE NITROGEN—TENTATIVE****26****PREPARATION OF SOLUTION**

Exhaust 7-25 g of the sample (depending upon the  $H_2O$  content) in the following manner: Weigh into a 150 cc beaker, add 5-10 cc of cold (15°)  $NH_3$ -free  $H_2O$ , and stir to a homogeneous paste. Add 50 cc of cold  $H_2O$ , stir for 15 min. at 3 min. intervals, let stand for 2-3 min., and decant the liquid thru a quantitative filter, collecting the filtrate in a 500 cc volumetric flask. Drain the beaker, pressing out the liquid from the meat residue by the aid of a glass rod. Add to the residue in the beaker 50 cc of cold  $H_2O$ , stir for 5 min., allow to stand 2-3 min., and decant as before. If a considerable portion of the meat is transferred to the filter, return it to the beaker by means of a glass rod. Repeat the extractions, using two 50 cc portions and four

25 cc portions of cold  $H_2O$ . After the last extraction transfer the entire insoluble portion to the filter and wash with three 10 cc portions of  $H_2O$ , allowing the material to drain thoroly after each addition of  $H_2O$ . Dilute to the mark and mix thoroly.

27

## DETERMINATION

Determine the total N in a 50 cc aliquot of the soln obtained under 26, proceeding as directed under II, 21, 23, or 25. Total N—soluble N=insoluble N.

28

## COAGULABLE NITROGEN—TENTATIVE

(For uncooked meat only.)

Measure 150 cc of the extract from 26 into a 250 cc beaker and evaporate to 40 cc on a steam bath, stirring occasionally. Neutralize to phenolphthalein, add 1 cc of 0.1 N acetic acid, and boil gently for 5 min. (The coagulum should separate at once, leaving a clear liquid.) Filter thru a quantitative paper and wash the beaker thoroly 4 times with hot  $H_2O$ , taking special care to clean the sides. Finally wash the coagulum on the filter 3 times; dilute the combined filtrate and washings to a definite volume; and reserve for the determination of proteose, peptone, and gelatin, 29, and creatin, 31. Transfer the coagulum with the paper to a Kjeldahl flask and remove, with  $H_2SO_4$ , any of the material adhering to the beaker, taking the usual 25 cc of acid in 5 cc portions for this purpose, heating the acid in the beaker on a hot plate, and rubbing with a glass rod. Proceed as directed under 8.

## PROTEOSE, PEPTONE, AND GELATIN NITROGEN

29

*Modified Tannin-Salt Method<sup>12</sup>—Tentative*

Transfer a 50 cc aliquot of the filtrate obtained under 28 to a 100 cc volumetric flask, add 15 g of NaCl and 10 cc of cold  $H_2O$ , shake until the NaCl has dissolved, and cool to 12°. Add 30 cc of 24% tannin soln cooled to 12°, dilute to the mark with  $H_2O$  previously cooled to 12°, shake, and allow the mixture to stand at a temp. of 12° for 12 hours, or overnight. Filter at 12°, transfer 50 cc of the filtrate to a Kjeldahl flask, and add a few drops of  $H_2SO_4$ . Place the flask in a steam bath, connect with a vacuum pump, and evaporate to dryness. Determine N in the residue as directed under II, 21, using 30 cc of  $H_2SO_4$  for the digestion. Conduct a blank determination, using the same quantity of reagents, and correct the result accordingly. Multiply the corrected result by 2 and deduct the quantity of N found from the N determined in another 50 cc aliquot of the filtrate from the coagulable N, 28, without the tannin-salt treatment; the difference  $\times 6.25$  = the percentage of proteose, peptone, and gelatin.

30

## MEAT BASES—TENTATIVE

Deduct from the percentage of total N, 7, the sum of the percentages of N obtained in the determination of insoluble N, 27, coagulable N, 28, and proteose, peptone, and gelatin, 29, to obtain the percentage of N of the meat bases. Multiply the result by 3.12 to obtain the percentage of meat bases.

31

## CREATIN—OFFICIAL

Evaporate an aliquot or the remaining portion of the filtrate and washings from the coagulable N, 28 (a portion having been used in 29) to 5–10 cc; transfer with a minimum quantity of hot  $H_2O$  to a 50 cc volumetric flask, keeping the volume below 30 cc; add 10 cc of 2 N HCl; and mix. Hydrolyze in an autoclave at 117–120° for

20 min., allow the flask to cool somewhat, remove, and chill under running  $H_2O$ . Partially neutralize the excess of acid by adding 7.5 cc of 10% NaOH soln, free from carbonates, dilute to the mark, and mix. Make a preliminary reading on 20 cc with a Duboseq colorimeter to ascertain the volume to use to obtain a reading of approximately 8 mm. Transfer such a volume of the soln to a 500 cc volumetric flask and add 10 cc of 10% NaOH soln and 30 cc of saturated picric acid soln (1.2%). Mix, rotate for 30 seconds, and let stand exactly 4.5 min. Dilute to the mark at once with  $H_2O$ ; shake thoroly; and read in a Duboseq colorimeter set at 8 mm, comparing the color with 0.5  $N$   $K_2Cr_2O_7$  soln.

If the reading is too high or too low (above 9.5 or below 7 mm), calculate the quantity necessary to obtain a reading of about 8 mm. The strength of the dichromate soln used must be checked against a standard creatin soln. Divide 81 by the reading and multiply by the volume factor to obtain the mg of creatinin; this value, multiplied by 1.16, gives creatin, which divided by the weight of the sample and multiplied by 100 gives the percentage of creatin.

The use of Kober's shade and the painting of the plunger, suggested for this nephelometer, assists in getting a sharper end point, relieves the eye strain, and may be used if desired.

EXAMPLE.—Twenty g of meat is extracted with  $H_2O$  as directed under 26, and the extract is diluted to 500 cc; 150 cc of this latter soln (equivalent to 6 g of meat) is treated as directed under 28. The filtrate thus obtained is then evaporated and hydrolyzed as above and diluted to 50 cc; 25 cc of this last soln is treated with NaOH soln and picric acid soln as directed above and diluted to 500 cc. This latter soln gives a Duboseq reading of 9 mm.

$$\frac{81}{9} \times \frac{50}{25} = \text{mg of creatinin}; \frac{0.018 \times 1.16 \times 100}{6} = 0.35\% \text{ creatin.}$$

#### AMINO NITROGEN

*Van Slyke Method*<sup>13</sup>—*Tentative*

#### 32

##### APPARATUS

Use the apparatus shown in Figs. 31 and 32, the former illustrating the manner in which the entire apparatus is arranged and the latter showing the details of the deaminizing bulb and connections. The Hempel gas pipet is filled with a soln containing 50 g of  $KMnO_4$  and 25 g of KOH per liter.

#### 33

##### DETERMINATION

Fill with  $H_2O$  the buret ( $F$ ), the capillary tube leading to the Hempel pipet, and also the other capillary as far as  $c$ . Introduce into  $A$  sufficient glacial acetic acid to fill  $\frac{1}{2}$  of  $D$ , etching the tube  $A$  with a mark to measure this quantity. Allow the acid to run into  $D$ , and turn cock  $c$  so as to allow the air to escape from  $D$ . Pour  $NaNO_2$  soln (300 g per liter) into  $A$  until  $D$  is filled and enough excess is present to rise a little above the cock into  $A$ .  $A$  is also marked for measuring off this quantity. Then close the gas exit from  $D$  at  $c$ , and  $a$  being open, shake  $D$  for a few seconds until the liquid is forced down to the 20 cc mark in  $D$ . Then close  $a$ , open  $c$ , and shake the apparatus rapidly with the motor for 2 min., these operations being for the purpose of expelling all the air from  $D$ . Then turn  $c$  and  $f$  so that  $D$  and  $F$  are connected.

Measure off in  $B$  10 cc or less, as the case may be, of the soln of the sample containing not more than 20 mg of amino N (about 1–2 g of the sample in the case of meat extracts) and allow it to run into  $D$ . Connect  $D$  with the motor as shown in Fig. 31 and shake for 5 min.

If the soln of the sample is viscous and threatens to foam over, rinse out *B*, and then thru it introduce a little caprylic alcohol into *D*, or if it is known beforehand that the sample will cause excessive foaming, introduce a little caprylic alcohol into *D* thru *B*, rinsing *B* with alcohol and ether or drying with a roll of filter paper before adding the soln of the sample.

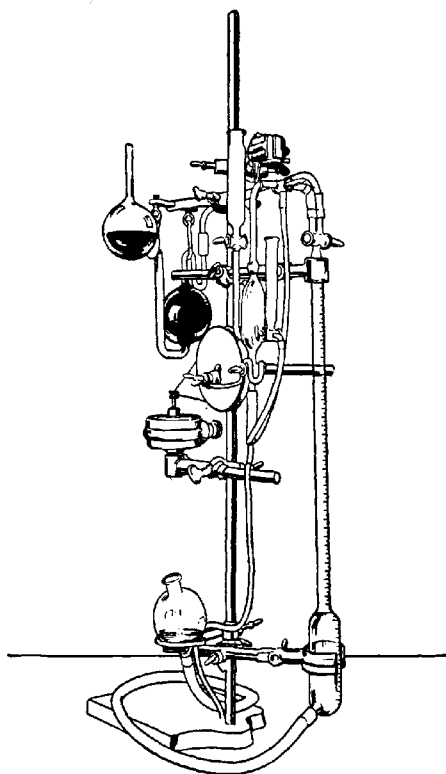


FIG. 31.—VAN SLYKE APPARATUS FOR THE DETERMINATION OF AMINO NITROGEN

During the shaking there is an evolution of  $N$  mixed with  $NO$ , the gases being collected in *F*. Force all the gas in *D* into *F* by opening *a* and filling *D* with liquid from *A*. Connect *F* with the Hempel pipet and force the gas into the latter by means of the leveling bulb, allowing the cock *a* to remain open during this and the succeeding operation in order to permit displacement of the liquid in *D* by the  $NO$  formed in the interval. Connect the driving rod with the pipet by lifting the hook from the

shoulder of *D* and placing the other hook, on the opposite side of the driving rod, over the horizontal lower tube of the pipet. Shaking the pipet rather slowly for a few min. completes the absorption of NO except with almost completely exhausted permanganate solns. Return the gas to the buret; adjust the level with the leveling bulb; note the volume of N, the temp., and the barometric pressure; and calculate the volume of N under standard conditions of temp. and pressure. Obtain the corresponding weight of N, divide the latter by 2, and from the quotient cal-

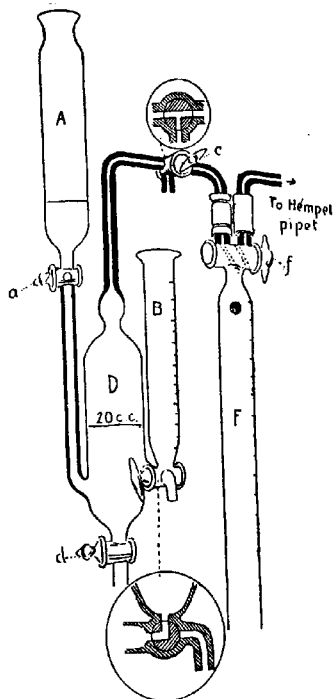


FIG. 32.—DETAILS OF THE DEAMINIZING BULB AND CONNECTION

culate the apparent percentage of amino N in the sample. Correct the result for a blank test performed as above, using 10 cc of  $H_2O$  instead of the soln of the sample. The quantity of gas obtained in the blank is usually 0.3–0.4 cc, and nitrite solns giving a much larger correction should be rejected.

With beef extracts and similar preparations, 5 min. is sufficient time to allow for the completion of the reaction in *D*. In general, the same time serves for the decomposition of alpha-amino acids, but with ammonia, methylamine, and most amines other than alpha-amines 1–1.5 hours should be allowed. For determinations on such



substances mix the soln of the sample with the reagents, as described above, allow the mixture to stand in the apparatus till the end of the required time, and conclude the reaction by shaking the apparatus with the motor for 2-3 min. Continue the determination from this point as directed above.

34

*Sørensen Method*<sup>14</sup>—*Tentative*

To 20 cc of the filtrate from 23, neutralized to phenolphthalein with  $\text{Ba}(\text{OH})_2$ , or  $\text{NaOH}$ , or to 20 cc of an equivalent extract of the meat (in some cases a larger volume may be necessary) add 10 cc of a freshly prepared phenolphthalein-formol mixture [50 cc of commercial formol containing 1 cc of a 0.5% soln of phenolphthalein in 50% alcohol, exactly neutralized with 0.2 *N*  $\text{Ba}(\text{OH})_2$  or  $\text{NaOH}$ ]. Titrate the mixture with 0.2 *N*  $\text{Ba}(\text{OH})_2$  soln until a distinct red color appears, add a slight known excess of 0.2 *N*  $\text{Ba}(\text{OH})_2$ , and titrate back to neutrality with 0.2 *N*  $\text{HCl}$ . Conduct a blank titration with the same reagents, using 20 cc of  $\text{H}_2\text{O}$  in place of the soln to be tested. From the quantity of 0.2 *N*  $\text{Ba}(\text{OH})_2$  required to neutralize the mixture, corrected for the quantity used in the blank titration, calculate the quantity of amino N present (including  $\text{NH}_3$  if this has not been removed). 1 cc of 0.2 *N*  $\text{Ba}(\text{OH})_2$  soln = 2.8 mg of amino N.

35

## TOTAL SOLUBLE PHOSPHORUS—TENTATIVE

Evaporate to dryness 50 cc of the water extract prepared under 26, moisten the residue with 10 cc of  $\text{H}_2\text{SO}_4$ , add a few drops of  $\text{HNO}_3$ , and heat on a hot plate until all the organic matter is destroyed. Add 100 cc of  $\text{H}_2\text{O}$ , boil for a few minutes, and proceed as directed under II, 9.

## 36 SEPARATION OF SOLUBLE INORGANIC AND ORGANIC PHOSPHORUS—TENTATIVE

To 500 cc of the extract prepared as directed under 26, add 50 cc of magnesia mixture, II, 7(c), and stir thoroly. Allow to stand 15 min., add 25 cc of  $\text{NH}_4\text{OH}$ , cover, and allow to stand 3 days. Filter, and wash the precipitate with  $\text{NH}_4\text{OH}$  (1+9). Dissolve the precipitate on the filter paper and that remaining in the beaker in dilute  $\text{HNO}_3$  (1+1) and hot  $\text{H}_2\text{O}$ , receiving the soln in a 400 cc beaker. Neutralize with  $\text{NH}_4\text{OH}$ , make slightly acid with  $\text{HNO}_3$ , add 5 g of  $\text{NH}_4\text{NO}_3$ , and determine P as directed under II, 9.

## MEAT EXTRACTS AND SIMILAR PRODUCTS

37

## PREPARATION OF SAMPLE—OFFICIAL

Remove liquid and semi-liquid meat extracts and similar preparations from the container and mix thoroly before sampling. (A little heating expedites the mixing of pasty extracts.) Carefully remove the sediment that forms in many liquid preparations from the bottom of the container and include in the sample. If the sample is in the form of cubes, grind 10-12 of the cubes in a mortar.

38

## MOISTURE—OFFICIAL

Proceed as directed under XXVII, 2, using about 2 g of powdered preparations, about 3 g of pasty preparations, and 5-10 g of liquid extracts, according to the solid content. Dry the powdered preparations directly without admixture. Dissolve the pasty preparations in  $\text{H}_2\text{O}$  and dry with sufficient ignited sand, asbestos, or pumice stone to absorb the soln. When glycerol is present, proceed as directed under XXVII, 6.

**39 ASH—OFFICIAL**

Proceed as directed under **XXVII, 8**. Add sufficient  $H_2O$  to pasty preparations to effect soln and evaporate to dryness in order that the solids may be distributed evenly over the bottom of the dish.

**40 TOTAL PHOSPHORUS—OFFICIAL**

Destroy organic matter as directed under **II, 8(c)** or **(d)**, and proceed as directed under **II, 9** or **12**.

**41 CHLORIDES—OFFICIAL**

Dissolve about 1 g of the prepared sample, **37**, in 20 cc of 5%  $Na_2CO_3$  soln and proceed as directed under **XII, 32, 33**.

**42 FAT—TENTATIVE**

Transfer the residue from the determination of moisture to a continuous extraction apparatus and proceed as directed under **XXVII, 22**.

**43 TOTAL NITROGEN—OFFICIAL**

Proceed as directed under **II, 21, 23** or **25**.

**44 AMMONIA—TENTATIVE**

Introduce 1 g of pasty extracts or 2–3 g of fluid extracts into tube *B* of the Folin apparatus and proceed as directed under **10**.

**45 INSOLUBLE NITROGEN<sup>15</sup>—TENTATIVE**

Dissolve in cold  $H_2O$  5 g of powdered preparations, 8–10 g of pasty extracts, and 20–25 g of fluid extracts. Filter, and wash with cold  $H_2O$ . Transfer the filter paper and contents to a Kjeldahl flask and determine N as directed under **II, 21, 23** or **25**. If a large quantity of insoluble matter is present, transfer the weighed sample to a volumetric flask, dilute to a definite volume, shake thoroly, filter thru a folded filter, and determine N in an aliquot of the filtrate. Total N, **43**, – N in the total filtrate = N in the insoluble N. Insoluble N  $\times 6.25$  = percentage of insoluble protein.

**46 COAGULABLE NITROGEN—TENTATIVE**

Prepare a soln of the sample as directed under **45**. Use as large an aliquot of the filtrate from the insoluble N, **45**, as practicable, and neutralize to phenolphthalein by the addition of acetic acid or NaOH, whichever may be necessary; add 1 cc of 1 N acetic acid, boil for 2–3 min., cool to room temp., dilute to 500 cc, and pass thru a folded filter.

Determine N in 50 cc of the filtrate as directed under **II, 21, 23** or **25**. Soluble N (total N – N occurring as insoluble N)  $- 10 \times$  the N obtained = the percentage of N present as coagulable N. Coagulable N  $\times 6.25$  = coagulable protein in the sample.

**47 PROTEOSES AND GELATIN<sup>14</sup>—TENTATIVE**

Evaporate the filtrate from **46** to a small volume and saturate with  $ZnSO_4$  (about 85 g to 50 cc, avoiding such an excess as would later cause bumping). Let stand several hours, filter, and wash the precipitate with saturated  $ZnSO_4$  soln. Place the filter and precipitate in a Kjeldahl flask and determine N as directed under **II, 21, 23** or **25**. Or, if the precipitate is voluminous, which is unusual, dilute to a definite volume with saturated  $ZnSO_4$  soln, filter, and determine the N in an aliquot of the

filtrate as directed under **II, 21, 23** or **25**. N in the filtrate from the coagulable N. **46**, —the N thus obtained = the N of the precipitated protein (proteoses and gelatin).

**48****GELATIN—TENTATIVE**

Prepare a 50% soln of the sample, using hot  $H_2O$ , allow to cool, and place in an ice box for 2 hours. If gelatin is present, the soln will set.

The ratio of total creatinin to total N in a normal meat extract (1:1.5) assists in determining the presence of gelatin or gelatin derivatives. The ratio is decreased when gelatin or gelatin derivatives are present in any considerable quantity.

**49****AMINO NITROGEN—TENTATIVE**

Proceed as directed under **33** or **34**, using an aliquot of the filtrate from **46**.

**50****ACID ALCOHOL-SOLUBLE NITROGEN—TENTATIVE**

Transfer 10 cc of an aqueous soln of the sample (10 g of the sample dissolved in sufficient  $H_2O$  to make 100 cc) or, if the sample is insoluble in  $H_2O$ , 1 g of the sample and 10 cc of  $H_2O$ , to a 200 cc glass-stoppered measuring cylinder; add 1.2 cc of 12% HCl, mix, and add absolute alcohol to the 200 cc mark. Mix thoroly and set aside for several hours. If necessary, make up to volume, filter, transfer 100 cc of the filtrate to a Kjeldahl flask, evaporate the alcohol on a water bath, and determine N in the residue as directed under **II, 21, 23** or **25**.

**51****CREATIN—OFFICIAL**

Dissolve about 7 g of the sample in cold ( $20^\circ$ )  $NH_3$ -free  $H_2O$  in a 150 cc beaker, transfer the soln to a 250 cc volumetric flask, dilute to the mark, and mix thoroly. Transfer a 20 cc aliquot of this soln to a 50 cc volumetric flask and proceed as directed under **31**. Subtract from the combined creatinin value the equivalent of the pre-formed creatinin, **52**, and multiply the difference by 1.16 to convert into creatin. Express the result as percentage of creatin.

**52****CREATININ—OFFICIAL**

Measure about 5 cc of the soln used in **51** into a 500 cc volumetric flask, add 10 cc of 10% NaOH soln and 30 cc of saturated picric acid soln (1.2%), mix, and rotate for 30 seconds. Allow to stand exactly 4.5 min. and then dilute to the mark at once with  $H_2O$ . Shake thoroly and read the depth of color after standing. If the reading is less than 7 or more than 9.5 mm, repeat, calculating the quantity of soln necessary to obtain a reading of about 8 mm. Express the result as percentage of creatinin, making the calculations as indicated under **31**.

**53****NITRATES (INCLUDING ALSO NITRITES)—TENTATIVE**

Proceed as directed under **13** or **15**.

**54****GLYCEROL<sup>14</sup>—TENTATIVE**

Weigh 2 g of a solid or 5 g of a liquid preparation in a small lead dish or thin glass shell containing 20 g of ignited sand. Transfer the dish and its contents to a mortar containing more ignited sand and several grams of anhydrous  $Na_2SO_4$  and mix thoroly. Transfer the mixture, including the dish, to a Soxhlet apparatus that has a piece of cotton placed in the side arm to prevent solid particles from being siphoned over. Extract the entire mass with redistilled anhydrous acetone for 10 hours. Distil the acetone from the extract, carefully removing the last trace by means of a

vacuum pump. Take up the residue in  $H_2O$ , add 5 cc of 10%  $AgNO_3$  soln, dilute to a volume of 100 cc, shake, allow to stand overnight, filter, and determine glycerol in an aliquot of the filtrate as directed under XXXIII, 72, beginning with "Add 1 cc of sulfuric acid." With solid meat and yeast extracts a blank of 0.5-1.0% is obtained in most cases.

55

## SUGAR—TENTATIVE

Heat 20 g of the sample with about 200 cc of  $H_2O$  on a steam bath until all soluble substances have gone into soln, and proceed from this point as directed under 22. Reducing sugars to the extent of 0.5% may be present as a natural constituent of meat extracts.

56

## PRESERVATIVES—OFFICIAL

Proceed as directed under XXXII.

57

## METALS—TENTATIVE

Proceed as directed under XXIX.

GELATIN<sup>19</sup>—TENTATIVE

58

## PREPARATION OF SAMPLE

In the case of ground gelatin mix thoroly. In the case of sheet gelatin break the sheets into small pieces by hand. Further comminution is unnecessary in either case.

59

## MOISTURE

Use a flat-bottomed metal dish approximately 55 mm in diameter and provided with a tightly fitting slip-in cover. Heat dish and cover to constant weight at  $100^\circ$ , cool, and weigh. Add approximately 2 g of the prepared sample, 58; cover loosely, and reweigh. Place the dish uncovered in a water-jacketed oven and dry for 6 hours at the temp. of boiling  $H_2O$ . Press the cover firmly in place, remove the dish from the oven, cool in a vacuum desiccator over  $H_2SO_4$ , and weigh. In releasing the vacuum admit the incoming air thru  $H_2SO_4$ . Report the loss in weight as moisture.

60

## ASH

Ignite at low redness, preferably in a muffle, as directed under XXVII, 8.

61

## TOTAL PHOSPHORUS

Treat the ash, 60, with 2-3 cc of  $HNO_3$  and evaporate to dryness on a steam bath. Repeat the  $HNO_3$  treatment and evaporation, take up the residue in hot  $H_2O$  containing a few drops of  $HNO_3$ , and proceed as directed under II, 9.

62

## NITROGEN

Proceed as directed under II, 21, 23 or 25, using a weighed quantity (about 2 g) of the sample. In the Kjeldahl and Gunning methods digest with the  $H_2SO_4$  for at least 4 hours after the mixture has become clear and in the Kjeldahl-Gunning-Arnold method for 2 hours after the mixture has become clear.

63

ARSENIC<sup>20</sup>

Heat 20 g of the sample with 75 cc of As-free  $HCl$  (1+3) in a covered vessel until all insoluble matter has flocculated and the gelatin is dissolved. Add an excess of Br water (about 20 cc) and neutralize with  $NH_4OH$ ; add 0.5 cc of 85%  $H_3PO_4$ , or

2 g of  $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ , or 2 g of crystallized  $\text{NaNH}_4\text{HPO}_4 \cdot 4\text{H}_2\text{O}$ ; and allow to cool. Precipitate the arsenic acid along with the phosphoric acid by adding an excess (about 30–35 cc) of magnesia mixture, II, 7(c). Allow to stand for 30 min., filter, wash the precipitate several times with  $\text{NH}_4\text{OH}$  (1+15), drain well, and dissolve in As-free  $\text{HCl}$  (1+3) to 50 cc volume in a volumetric flask. Take a 25 cc aliquot, dilute to 40 cc with  $\text{HCl}$  (1+3), and proceed as directed under XXIX, 4, beginning with "add 5 cc of the KI reagent." Use  $\text{HCl}$  in preparing the standard stains and run a blank determination on the reagents used. Arsenic impurities, if present, are usually found in the phosphate added.

## 64

## COPPER

Hydrolyze 50 g of the sample with 150 cc of  $\text{HCl}$  (1+3) as directed under 63, heating about 2 hours on a steam bath. To facilitate filtration and separation from Zn and Fe later, use the phosphoric acid or phosphate, and magnesia mixture, as directed under 63. Precipitate with  $\text{H}_2\text{S}$  in a slightly ammoniacal soln. Allow the precipitate to settle, filter, and wash with 5%  $\text{NH}_4\text{Cl}$  soln saturated with  $\text{H}_2\text{S}$ . Dissolve off the Zn and Fe sulfides,  $\text{MgNH}_4\text{PO}_4$ , etc., in 75 cc of  $\text{HCl}$  (1+9) saturated with  $\text{H}_2\text{S}$ . Reserve the soln for the determination of Zn as directed under 65.

Digest the filter and  $\text{CuS}$  with 4 cc of  $\text{H}_2\text{SO}_4$  and sufficient  $\text{HNO}_3$  until the residue fumes freely and remains perfectly colorless. Cool, and add 10 cc of  $\text{H}_2\text{O}$  and a slight excess of  $\text{Br}$  water. Boil off the excess of  $\text{Br}$ , cool, and make alkaline with  $\text{NH}_4\text{OH}$ . Filter to remove any  $\text{Fe}(\text{OH})_3$  or other precipitate formed and wash the filter and precipitate with hot  $\text{H}_2\text{O}$ . Remove excess of  $\text{NH}_3$  in filtrate by heating; then acidify with 1 cc of glacial acetic acid. Cool, add 1 g of KI, and titrate with 0.01 *N*  $\text{Na}_2\text{S}_2\text{O}_3$  soln with or without starch indicator. 1 cc of 0.01 *N*  $\text{Na}_2\text{S}_2\text{O}_3$  = 0.63 mg of Cu.

## 65

## ZINC

Boil the Zn and Fe soln under 64 to expel all  $\text{H}_2\text{S}$ . Make the soln decidedly ammoniacal and then make it decidedly acid with  $\text{HCOOH}$  (1+1). Filter while hot to remove insoluble matter as alumina, etc., and then pass in rapidly a stream of  $\text{H}_2\text{S}$  for 10 min. Warm the soln 15 min. on a steam bath; then remove and allow to stand for 30 min. before filtration. Filter, wash the precipitate of  $\text{ZnS}$  with 2%  $\text{NH}_4\text{CNS}$  soln, dry, ignite at the highest temp. of a Bunsen burner, cool, and weigh the  $\text{ZnO}$ .

## 66

POLARISCOPIC CONSTANTS<sup>11</sup>

Prepare a soln of a concentration of 3 g per 100 cc by soaking 3 g of the sample in 40–50 cc of cold  $\text{H}_2\text{O}$  for approximately 15 min., heating to complete soln at about 50° and diluting to a volume of 100 cc at 35°. Polarize at 35° in a 2 dm tube, using the Ventzke scale.

Cool a portion of the gelatin soln rapidly to 10–15° and pour into cold, dry 1 dm tubes before jelly has had time to form. Place the tube in a constant temp. bath at 15° for 18 hours to obtain equilibrium rotation, and then polarize at 15°. Double the reading to place it on the basis of a 2 dm tube.

In order to clarify cloudy samples before polarizing, digest the original 100 cc in a stoppered flask with approximately 10 g of lightly powdered  $\text{MgCO}_3$  for at least 1 hour at 35–40° and filter thru a folded filter until clear, avoiding unnecessary evaporation.

The increase in levorotation (mutarotation) between 35° and 15° is an index of the jelly strength developed.

## SULFUR DIOXIDE

67

*Distillation Method*

Proceed as directed under XXXII, 32.

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- <sup>3</sup> Ibid., **11**, 408 (1928).
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- <sup>5</sup> Tiemann, *Anleitung zur Untersuchung von Wasser*, 1870, p. 56; Wiley, *Principles and Practice of Agricultural Analysis*, 2nd ed., 1908, vol. 2, p. 397; U. S. Dept. Agr. Bur. Chem. Bull. **13** (X), p. 1403; J. Assoc. Official Agr. Chem., **4**, 502 (1921); **6**, 74 (1922).
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- <sup>18</sup> J. Assoc. Official Agr. Chem., **1**, 279 (1915).
- <sup>19</sup> Ibid., **5**, 343 (1922); J. Ind. Eng. Chem., **15**, 942 (1923).
- <sup>20</sup> U. S. Dept. Agr. Bur. Chem. Circ., **102**; J. Soc. Chem. Ind., **26**, 1115 (1907).
- <sup>21</sup> J. Assoc. Official Agr. Chem., **4**, 520 (1921).

## XXIX. METALS IN FOODS

### ARSENIC<sup>1</sup>

#### *Gutzeit Method—Official*

#### I

#### REAGENTS

(a) *Stannous chloride soln.*—Dissolve 40 g of As-free  $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$  in HCl and make up to 100 cc with the same strength acid.

(b) *Zinc.*—Use 20- or 30-mesh, As-free granulated Zn, which needs no preliminary treatment, or As-free stick Zn either cut into pieces 1 cm in length, preferably by means of a bolt clipper equipped with a securely mounted guide, or melted and cast into pellets of approximately equal size and surface area in a porcelain mold drilled 9 mm in diameter and 12.5 mm deep. Activate the pieces of Zn with HCl (1+3), to which has been added 2 cc of the  $\text{SnCl}_2$ , allowing the action to continue 15 min. Sort out distinctly inactive or overactive pieces and pour off the liquid. Wash the Zn free from acid with hot, distilled  $\text{H}_2\text{O}$ , or with clear running  $\text{H}_2\text{O}$ , and then rinse with hot, distilled  $\text{H}_2\text{O}$ . Select uniformly etched non-pitted Zn. To maintain a supply of uniform Zn when a large number of determinations are run, maintain a system of rotation, using the Zn from an original receptacle until the stock is exhausted and putting the used Zn into a second receptacle. Discard non-uniform pieces and use the Zn from the second receptacle after washing it with clear running  $\text{H}_2\text{O}$ . Repeat this procedure until the pieces are too small for further use.

(c) *Ammonium oxalate soln.*—Saturated.

(d) *Potassium iodide soln.*—Dissolve 15 g of KI in  $\text{H}_2\text{O}$  and dilute to 100 cc.

(e) *Sand.*—Clean 30-mesh (thru 30- but not 40-mesh) white sea sand by washing successively with hot 10% NaOH soln, hot concentrated  $\text{HNO}_3$ , and hot distilled  $\text{H}_2\text{O}$ . Dry the clean sand.

(f) *Mercuric bromide paper.*—Cut heavy, cold-pressed, close-textured drafting paper, similar to Whatman No. 40, into strips exactly 2.5 mm wide and about 12 cm long. (This paper can be purchased in individual strips or in sheets of cut strips. If purchased already cut, examine the strips carefully to see that they have been cut from paper of the same weight and texture and are uniform in width. Uniformity of width and character of paper are of first importance; slight variations may cause considerable difference in the length of stain produced by the same quantity of As. The Hanford-Pratt or similar As strips have proved to be very satisfactory.) To sensitize, soak the strips for 1 hour or longer in a 3–6% soln of  $\text{HgBr}_2$  in 95% alcohol, according to the quantity, character, and activity of the Zn used. If the strips are in sheets, cut off two sides before soaking and leave the strips attached at the ends. (The length and density of stain for any given strips are controlled primarily by the activity of the Zn and secondarily by the strength of the  $\text{HgBr}_2$ . A 5% soln of  $\text{HgBr}_2$  is satisfactory in most instances.) Have the sensitizing soln of  $\text{HgBr}_2$  in a fresh condition—in no case use it after a precipitate begins to deposit. After sensitization remove the strips and dry the individual ones on glass rods and the groups by waving them in the air. (If desired, place the strips when nearly dry between clean sheets of paper and subject them to pressure long enough to take out any bends or curls.) When the individual strips are used, cut them off squarely about half an inch from one end and insert this end into the narrow tube. Handle the strips only at one end, keeping the rest of the strip clean and free of any contamination. Compare stains only with a curve obtained from standard strips run at the

same time and from the same strip-group. (Aging of the impregnated strips usually results in markedly fainter and longer stains. The most desirable type of stain results from the use of strips that have not been impregnated longer than 2 days.)

(g) *Standard arsenic soln.*—Dissolve 1 g of  $\text{As}_2\text{O}_3$  in 25 cc of 20% NaOH. Saturate the soln with  $\text{CO}_2$  and dilute to 1 liter with recently boiled  $\text{H}_2\text{O}$ . 1 cc of this soln contains 1 mg of  $\text{As}_2\text{O}_3$ . Dilute 40 cc of this soln to 1 liter. Make 50 cc of the diluted soln to 1 liter and use to prepare the standard stains. 1 cc of the latter soln contains 0.002 mg of  $\text{As}_2\text{O}_3$ . A soln containing 0.001 mg of  $\text{As}_2\text{O}_3$  may also be prepared if desired. Prepare fresh dilute solns at frequent intervals.

## 2

## APPARATUS

(a) *Generators and absorption tubes.*—Use 2 oz. wide-mouthed bottles of uniform capacity and design as generators, and fit each by means of a perforated stopper with a glass tube 1 cm in diameter and 6–7 cm long, with an additional constricted end to facilitate connection. Place a small wad of glass wool in the constricted bottom end of the tube and add 3.5–4 g of the 30-mesh cleaned sand, taking care to have the same quantity in each tube. Moisten the sand with 10% Pb acetate soln and remove excess by light suction. Clean the sand when necessary by treatment (do not remove sand from tube) with  $\text{HNO}_3$  followed by an  $\text{H}_2\text{O}$  rinse and suction. Treat with the Pb acetate soln. If the sand has dried thru disuse, clean and re-moisten it as directed. Connect the tube by means of a rubber stopper with a narrow glass tube 2.6–2.7 mm in internal diameter and 10–12 cm long, and introduce the clean end of the strip of  $\text{HgBr}_2$  paper. (A 3 mm bore allows the strip to curl, which results in an uneven stain and a poor end point.) Clean and dry the tube before inserting the bromide paper. An ordinary pipe cleaner may be used.

(b) *Water bath.*—Use any constant temp. water bath. If no water bath is available, use any flat-bottomed container of suitable depth and capacity. (A deep water bath is suggested to insure uniform conditions during evolution and absorption of the As.)

## 3

## PREPARATION OF SAMPLE

(a) *For fresh fruits (apples, pears or similar products).*—Weigh and peel a representative sample of the fruit (1–5 lbs.). At the blossom and stem ends cut out all flesh thought to be contaminated with arsenical compounds and include with the peelings. Place the peelings in 1 or more 800 cc Pyrex Kjeldahl flasks. (As-free Pyrex glassware and “wet ashing” apparatus of Duriron are now available.) Add 25–50 cc of  $\text{HNO}_3$ , then add cautiously 20 cc of  $\text{H}_2\text{SO}_4$ . Place each flask on an asbestos mat with a 2 in. hole. Warm slightly and discontinue heating if foaming becomes excessive. When the reaction has quieted, heat cautiously and rotate the flask from time to time to prevent caking of the sample upon the glass exposed to the flame. Maintain an oxidizing mixture in the flask at all times during the digestion by adding cautiously small quantities of  $\text{HNO}_3$  whenever the mixture turns

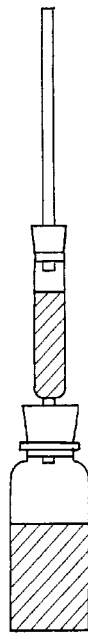


FIG. 33.—  
GENERATOR TO  
BE USED WITH  
THE GUTZEIT  
METHOD FOR  
THE DETERMI-  
NATION OF AR-  
SENIC



brown or darkens. Continue the digestion until the organic matter is destroyed and  $\text{SO}_2$  fumes are copiously evolved. The final soln should be water-white, or at most a light straw color. Cool slightly and add 75 cc of  $\text{H}_2\text{O}$  and 25 cc of the saturated soln of  $\text{NH}_4$  oxalate to assist in expelling oxides of N from the soln. Evaporate again to the point where fumes of  $\text{SO}_2$  appear in the neck of the flask. Cool, and dilute with  $\text{H}_2\text{O}$  to 500 or 1000 cc in a volumetric flask.

(b) *For dried fruit products.*—Prepare the sample by alternately grinding and mixing 4–5 times in a food chopper. Place 35–70 g portions in 800 cc Kjeldahl flasks, and add 10–25 cc of  $\text{H}_2\text{O}$ , 25–50 cc of  $\text{HNO}_3$ , and 20 cc of  $\text{H}_2\text{SO}_4$ . Continue the digestion as directed in 3(a). Dilute the digested soln to 250 cc.

(c) *For small fruits, vegetables, etc.*—Use 70–140 g of sample and digest as directed under 3(a) and (b).

(d) *For materials other than (a), (b), or (c).*—Digest 5–50 g, according to degree of dryness and amount of As expected, as directed under 3(a) and (b). Dilute to definite volume dictated by circumstances.

(e) *For products containing stable organic As compounds, products liable to yield incompletely oxidized organic derivatives that inhibit arsine evolution, or products that are especially difficult to digest.*—Shrimp, tobacco, and oils, and possibly other products, require special treatment previous to the As determination. For details consult the following references:

(1) C. R. Gross, *Ind. Eng. Chem. Anal. Ed.*, **5**, 58 (1933). Pyridine residues from nicotine in tobacco inhibit arsine evolution. Arsenic can be isolated from pyridine and other interfering substances by co-precipitation of magnesium ammonium phosphate and arsenate.

(2) Carey, Blodgett, and Satterlee, *Ind. Eng. Chem. Anal. Ed.*, **6**, 327 (1934). Dry samples are oxidized in an oxygen bomb, and the contents of the bomb are treated in a special apparatus.

(3) Remington, Coulson, and von Kolnitz, *Ind. Eng. Chem. Anal. Ed.*, **6**, 280 (1934). Samples are burned in an enclosed torch and products of combustion are washed in an absorption train.

Dilute the As solns obtained by these special methods of preparation to definite volume.

## 4

## ISOLATION OF ARSENIC

Whenever it is desirable to concentrate the As, when interfering substances are present in the digests (pyridine), or when samples contain excessive amounts of salts, or  $\text{H}_2\text{SO}_4$  from digestions, isolate the As before making the determination. Consult reference 3(1), or use the trichloride distillation of As, bromate method, 6.

## 5

## DETERMINATION

Determine the acid ( $\text{HCl}$  or  $\text{H}_2\text{SO}_4$  according to the previous treatment), by titration if necessary, in a definite volume of the sample soln. Place aliquots (not to exceed 30 cc) in Gutzeit generators, gaging the quantity of As in the aliquot to 0.01–0.03 mg of  $\text{As}_2\text{O}_3$  (0.020–0.025 mg is optimum). If the arsenic in the aliquot taken is found to be outside the limits specified, repeat with the proper aliquot. If the aliquot contains only  $\text{HCl}$ , add sufficient  $\text{HCl}$  to make a total volume of 5 cc; if it contains  $\text{H}_2\text{SO}_4$ , add sufficient 25% As-free  $\text{NaOH}$  soln (keep in As-free Pyrex) to exactly neutralize it and add 5 cc of  $\text{HCl}$ , or add sufficient  $\text{HCl}$  to the  $\text{H}_2\text{SO}_4$  in the aliquot to make a total volume of 5 cc. Cool when necessary and add 5 cc of the  $\text{KI}$  reagent and 4 drops of the  $\text{SnCl}_2$ , 1(a). From the standard As soln prepare

standards corresponding to 0.010, 0.020, and 0.030 mg of  $\text{As}_2\text{O}_3$ . As the standards must contain the same kind and amounts of acid as the samples, add 5 cc of  $\text{HCl}$ , or  $\text{H}_2\text{SO}_4$ , and  $\text{HCl}$  (total 5 cc) according to the prior treatment of the unknown. If the  $\text{H}_2\text{SO}_4$  has been neutralized, add an equivalent quantity of As-free  $\text{Na}_2\text{SO}_4$  to the standards. Mix, and allow to stand for 30 min. at not less than  $25^\circ$  or 5 min. at  $90^\circ$ . Dilute with  $\text{H}_2\text{O}$  to 40 cc.

Prepare the generator as directed under 2 and center a strip of  $\text{HgBr}_2$  paper carefully in the narrow tube. According to the activity of the Zn, add to each of the standards and samples 10–15 g of activated stick Zn or 2–5 g of granulated Zn and add the same quantity to each generator. Equalize as far as possible the surface area of Zn exposed in standard and sample.

Immerse the apparatus to within 1 inch of the top of the narrow tube in the water bath, which is kept at a constant temp. between  $20$  and  $25^\circ$ , and allow the evolution to proceed for 1.5 hours. Remove the strip and average the length of the stains on both sides in mm. Plot a graph of the standard strips on cross-sectioned paper, using the length in mm as ordinates and the mg of  $\text{As}_2\text{O}_3$  as abscissa. (The preparation of a standard graph averages the errors of individual standards. Reading the strip from such a graph is considered more convenient and accurate than comparing the strips themselves.) Locate the length of the unknown strip on a standard graph and read off on the abscissa the quantity of As present. Report only to the third decimal as grains of  $\text{As}_2\text{O}_3$  per pound. Take smaller or larger aliquots when the stain is longer or shorter than the highest or lowest standard, respectively. Grain/lb.  $\times 143 = \text{p.p.m.}$ ;  $\text{p.p.m.} \times 0.007 = \text{gr./lb.}$

Frequent blanks should be made. With reagents of suitable quality, blanks should not show more than 0.001 mg of  $\text{As}_2\text{O}_3$ .

#### Bromate Method<sup>2</sup>—Tentative

(Applicable to the determination of arsenic in plants and food products where a sample of convenient size for digestion will yield at least 0.005 grain (0.324 mg) of  $\text{As}_2\text{O}_3$ .)

#### 6

##### REAGENTS

(a) *Ammonium oxalate-urea soln.*—To a saturated  $\text{H}_2\text{O}$  soln of ammonium oxalate add 50 g of urea per liter.

(b) *Hydrazine sulfate-sodium bromide soln.*—Dissolve 20 g of hydrazine sulfate and 20 g of  $\text{NaBr}$  in 1 liter of  $\text{HCl}$  (1+4).

(c) *Sodium chloride.*—Commercial salt, uniodized.

(d) *Standard potassium bromate soln.*—Dissolve 0.1823 g in  $\text{H}_2\text{O}$  and dilute to 1 liter. 1 ml = 0.005 grain of  $\text{As}_2\text{O}_3$ . Standardize by titration against the standard arsenious oxide soln, (e), making the titration at about  $90^\circ$  and in the presence of about 100 ml of  $\text{H}_2\text{O}$  and 25 ml of  $\text{HCl}$ , in order to simulate the conditions under which the samples will be titrated. 1 ml of the bromate soln should be equivalent to 1 ml of the  $\text{As}_2\text{O}_3$  soln.

(e) *Standard arsenious oxide soln.*—Dissolve 0.3241 g of  $\text{As}_2\text{O}_3$  in 25 ml of 10%  $\text{NaOH}$ , make slightly acid with  $\text{H}_2\text{SO}_4$  (1+6), and dilute with  $\text{H}_2\text{O}$  to 1 liter.

#### 7

##### DISTILLING APPARATUS

The distilling apparatus consists of an 800 ml Kjeldahl flask (A), distilling tube (B), and 300 ml Erlenmeyer flask (C).

To prepare the distilling tube, bend a 10–15 mm glass tube to an acute angle of

about 70°. Draw the longer arm, which is about 15–20 in. long, down to an orifice of about 3 mm. Fit the shorter arm (about 4 in.) with a No. 7 rubber stopper, which has previously been boiled in 10% NaOH for 15 min., and then in HCl for 15 min., in order to remove most of the sulfur compounds which might be distilled and react with the bromate soln.

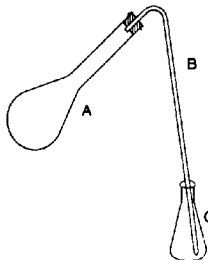


FIG. 31.—DISTILLING APPARATUS FOR THE DETERMINATION OF ARSENIC BY THE BROMATE METHOD

## 8

## PREPARATION OF SAMPLE

Introduce a suitable sample, 3, containing 0.005 grain (0.324 mg) or more of  $\text{As}_2\text{O}_3$  into an 800 ml Kjeldahl flask. Proceed with the acid digestion as directed under the Gutzzeit method with the following exception: Add exactly 20 ml of  $\text{H}_2\text{SO}_4$ , or (rarely), if the material is difficult to digest, exactly 25 ml at the beginning of the digestion. After digestion is complete, add 50 ml of  $\text{H}_2\text{O}$  and 25 ml of the ammonium oxalate-urea soln, and boil until white  $\text{SO}_2$  fumes extend up into the neck of the flask to decompose oxalates and urea completely. (Volatile intermediate products may titrate with bromate. If the heat available is insufficient to decompose these substances, it is preferable to evaporate to fumes with  $\text{H}_2\text{O}$  alone. Hydrazine sulfate will destroy small amounts of oxides of nitrogen.)

## 9

## ISOLATION

Add 25 ml of  $\text{H}_2\text{O}$  to the digested soln in the Kjeldahl flask and cool to room temp. Put 100 ml of  $\text{H}_2\text{O}$  into the Erlenmeyer flask. Add to the soln in the Kjeldahl flask 20 g of NaCl and 25 ml of the hydrazine sulfate-sodium bromide soln and connect the distilling tube. Heat the Kjeldahl flask over a small well-protected flame, and distil into the  $\text{H}_2\text{O}$  in the Erlenmeyer flask. (The heating is not intended to boil the soln but to bring about the evolution of the HCl gas, which carries over the  $\text{AsCl}_3$  with it. The absorption of the evolved HCl gas by the  $\text{H}_2\text{O}$  causes a rise in temp., which furnishes an indication of the progress of the distillation.) Adjust the flames so that the temp. of the distillate soln will rise to 90° in 9–11 min. and then discontinue distillation. (The residual mixture in the flask should not be less than 55 ml.) If the distillation proceeds further, or a larger quantity of  $\text{H}_2\text{SO}_4$  than that specified is used in the digestion,  $\text{SO}_2$  is distilled, which is titrated as As.

## 10

## DETERMINATION

Titrate the distillate at once with the bromate soln, using 3 drops of methyl orange indicator. Single drops of indicator, but not exceeding 3, may be added during titration as the red color fades. Towards the end of the titration add the

bromate soln very slowly and with constant agitation to prevent local excess. The end point is reached when a single drop of the bromate just destroys the final tinge of red color. To determine when this point has been reached, use an Erlenmeyer flask of clear  $H_2O$  for comparison. The end point must not be exceeded as the action of the indicator is not reversible and back titrations are not reliable. At the proper end point, the red color produced by 2 additional drops of methyl orange indicator should persist for at least 1 min. Correct results for the volume of bromate used in a blank run (digest 5 g of pure sucrose) with the same reagents (same quantities) and the regular distillation procedure. The blank titration should not exceed 0.7 ml of bromate soln. The method is accurate down to the variations in the blank, which should not exceed 0.1 ml when chemicals from the same lot are used. Should the blank titration be high or variable, test the individual reagents for purity by bromate titration and discard unsatisfactory ones. Test the  $H_2SO_4$  by bringing 20 ml to a boil, cooling, diluting with  $H_2O$  to 100 ml, adding a little  $HCl$ , and titrating while hot. It probably will furnish most of the blank. Select rubber stoppers carefully as they are often the source of unsatisfactory blanks.

If high results, due to  $SO_2$  produced during distillation, or other reducing substances, are suspected, dilute the titrated distillate to a definite volume and re-determine the As in an aliquot by the Gutzeit method. A positive test for sulfates in an aliquot of the titrated distillate indicates contamination with reduced sulfur compounds and a necessity for a check on the As.

#### LEAD—TENTATIVE

##### 11

###### PRINCIPLES

The general method calls for ashing, 14, separation of the Pb, either as the dithizone complex, 16, or as the sulfide, 17, followed (depending upon the quantity) by electrolytic determination, 18, 19, 20, or by colorimetric dithizone determination in comparator tubes, 22, or with a photometer, 23. At various points in the procedure provision is made for removal of contaminating elements, such as Sn and Bi. The subject of interference is treated separately, 24–26, and the analyst should familiarize himself with the details of these sections before applying the method. Special methods of preparation are presented under 27–30. The continuity of procedure and the interrelationships of methods for sample preparation with those for preliminary separation of Pb and its final isolation from interferences are summarized in a "flow sheet" diagram, Fig. 35. The heavy solid lines represent the preferred procedures. Those used infrequently are indicated in lighter solid lines. The dotted lines indicate procedures adapted for speed (approximately 95% recoveries are expected).

##### 12

###### PRECAUTIONS

The analyst should decide whether the nature of the determination requires unusual precautions in purification of reagents, or whether a blank determination will be sufficient. The smaller the quantity of Pb to be determined, the greater the care required in the reduction of the blank (see also 21).

To test the suitability of any reagent place 15–20 cc of concentrated acids or 10–15 g of solid reagents dissolved in redistilled  $H_2O$  in a separatory funnel and add sufficient Pb-free citric acid to prevent precipitation by ammonia of iron, aluminum, alkaline earth phosphates, or other substances. Make the soln ammoniacal and add 2–3 cc of 10%  $KCN$ . Shake the soln with about 5 cc of dithizone soln, 13(e) (5–10 mg per liter). If the lower layer is green, transfer it to another separatory funnel and



extract the excess dithizone with  $\text{NH}_4\text{OH}$  (1+99) to which has been added a drop of KCN soln. If the  $\text{CHCl}_3$  layer is colorless, consider the test negative for general analytical purposes.

When special purification becomes necessary, redistill  $\text{H}_2\text{O}$  (distilled  $\text{H}_2\text{O}$  stored in tin-lined tanks usually contains Pb and Sn),  $\text{NH}_4\text{OH}$ ,  $\text{HNO}_3$ ,  $\text{HCl}$ ,  $\text{HBr}$ ,  $\text{Br}$ , and  $\text{CHCl}_3$  (U.S.P. free from chlorides) in resistant, all-glass stills (Pyrex is suitable). If the stills are new, steam them out with hot  $\text{HCl}$  or  $\text{HNO}_3$  vapors to remove "surface" Pb. (Subsequent distillates may not be totally Pb-free.) Purify citric acid, Na or  $\text{NH}_4$  acetate,  $\text{Al}(\text{NO}_3)_3$ ,  $\text{Ca}(\text{NO}_3)_2$ , and  $\text{Na}_2\text{SO}_4$  by precipitating the Pb from their aqueous solns with  $\text{H}_2\text{S}$ , using 5–10 mg of  $\text{CuSO}_4$  as a coprecipitant (citric acid and aluminum nitrate solns require adjustment with ammonia to pH 3.0–3.5, bromophenol blue indicator). Filter (a fritted glass filter is most convenient), boil the filtrates for 20 min. to expel excess  $\text{H}_2\text{S}$  and filter again if necessary to obtain brilliantly clear solns. Purify other reagents by recrystallization.

Store redistilled acids or purified solns of reagents in resistant glass containers of minimum Pb content (Pyrex is suitable) carefully cleaned of surface Pb with hot  $\text{HNO}_3$ .

Clean new glass and chemical ware carefully with hot 10%  $\text{NaOH}$  soln followed by hot  $\text{HNO}_3$  and use only for Pb determinations.

In the preparation of samples for analysis, avoid Pb contamination. If mixing or grinding is necessary, use a porcelain mortar if possible. Avoid the use of metal food grinders unless previous experiment has shown that no contamination of the sample with Pb or Sn results. If the product to be analyzed cannot be thoroly mixed in its own container, or if a composite sample of a number of containers is desired, empty into a large glass jar or porcelain dish and mix thoroly with a wooden spoon or porcelain spatula. If the liquid portion of the sample cannot be incorporated into the ground solid material to obtain a homogeneous mixture, analyze separately. If the food is packed in tins having soldered seams (sardines and meats), open the tins from the bottom to avoid contaminating the sample with bits of solder. Avoid sifting in the preparation of samples to prevent metallic contamination or segregation of Pb.

## GENERAL METHOD

### *Sn and Bi Absent*

(Applicable to such materials as carbohydrates, cereals and cereal products, cacao and dairy products, feeds, meats, fish, plant material, fruit and fruit products, fresh vegetables, etc., and in general to all organic materials (except fats) in which no Sn and Bi are encountered. For products containing Sn (canned foods) or Bi, proceed as directed under 24–26.)

## 13

### REAGENTS

(a) *Standard lead solns.*—Dissolve 20–50 g of C.P. Pb nitrate in a minimum of hot  $\text{H}_2\text{O}$  and cool with stirring. Filter the crystals with suction on a small Büchner funnel, redissolve, and repeat the recrystallization. Dry the crystals at 100–110° to constant weight. Cool in a desiccator and preserve in a tightly stoppered bottle. (The product has no water of crystallization and is not appreciably hygroscopic.) Prepare a stock soln containing the equivalent of 2 mg of Pb (3.197 mg of  $\text{Pb}(\text{NO}_3)_2$  per cc in 1%  $\text{HNO}_3$ ) (b). Prepare weaker dilutions with 1%  $\text{HNO}_3$  as needed, and do not store over long periods, because Pb tends to precipitate out.

(b) *Nitric acid.*—1%. Dilute 10 cc of fresh, water-white  $\text{HNO}_3$  (sp. gr. 1.40) to

1 liter with redistilled  $\text{H}_2\text{O}$ . (If the acid has been redistilled, boil off nitrous fumes and readjust to 1.40 sp. gr. by evaporation or dilution.)

(c) "*Ash Aid*" soln.—Dissolve 40 g of  $\text{Al}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$  + 20 g of  $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$  in 100 cc of  $\text{H}_2\text{O}$ .

(d) *Citric acid*.—Concentrated Pb-free soln. 1 cc = 0.5 g of citric acid.

(e) *Diphenylthiocarbazone (dithizone)*.—Dissolve about 1 g of the commercial reagent in 50–75 cc of  $\text{CHCl}_3$  and filter if insoluble material remains. Shake out in a separatory funnel with four 100 cc portions of metal-free (redistilled) ammonia (1+99). Dithizone passes into the aqueous phase to give an orange colored soln. Filter the aqueous extracts into a large separatory funnel thru a pledget of cotton inserted in the stem of a funnel. Acidify slightly with dilute HCl and extract the precipitated dithizone with two or three 20 cc portions of  $\text{CHCl}_3$ . Combine the extracts in a separatory funnel and wash two or three times with  $\text{H}_2\text{O}$ . Draw off into a beaker and evaporate the  $\text{CHCl}_3$  with gentle heat on the steam bath, avoiding spattering as the soln goes to dryness. Remove the last traces of moisture by heating for an hour at not over  $50^\circ$  in vacuo. Store the dry reagent in the dark in a tightly stoppered bottle. Make up the reagent solns for extraction to contain approximately 100, 50, and 10 mg per liter in redistilled  $\text{CHCl}_3$ . A stock soln of dithizone in  $\text{CHCl}_3$  containing 1 mg per cc will keep a long time and is convenient for use in making dilutions.

(f) "*Stripping*" reagent.—To 20 cc of saturated Na acetate soln, add 10 cc of glacial acetic acid and make to 100 cc.

(g) *Potassium iodide soln*.—2%. Prepare as frequently as is necessary to prevent formation of a starch-iodine color when mixed with reagent (f) in the proportions specified in 20.

(h) *Starch soln*.—Make up 1 g of *Soluble starch* to 200 cc.

(i) *Sodium thiosulfate*.—Approximately 0.1 *N* stock soln. Dissolve 24.8 g of  $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$  in 1 liter of  $\text{CO}_2$ -free  $\text{H}_2\text{O}$  and allow it to stand (preferably for 2 weeks) before use. Prepare approximately 0.001 and 0.005 *N* solns by dilution of the stock soln in the exact ratio of 1:100 or 1:20 with  $\text{CO}_2$ -free  $\text{H}_2\text{O}$  and standardize these electrolytically, using standard Pb soln equivalent to 0.2–1.0 mg of Pb for the 0.001 *N* dilution and 1–5 mg of Pb for the 0.005 *N* dilution. Subtract anode blanks, 19–20, and take as the thiosulfate factor the average number of mg of Pb equal to 1 cc of the solns. Make fresh dilutions daily and check the Pb factor at least every month.

(j) *Ammonia-cyanide mixture*.—To 100 cc of 10% KCN or NaCN in a 500 cc volumetric flask add sufficient redistilled ammonia soln to introduce 19.1 g of  $\text{NH}_3$  and complete to volume with redistilled  $\text{H}_2\text{O}$ . (Strength of redistilled ammonia can be determined by sp. gr. or titration.)

(k) *Pure metallic tin*.—Purest obtainable, such as Bureau of Standards Sample No. 42 B (0.0035% Pb). Granulate the tin as finely as possible by melting and pouring very slowly into  $\text{H}_2\text{O}$ . Determine the Pb content as follows. Dissolve a 1–2 g sample in HBr or HCl and volatilize the Sn by evaporating the soln to dryness and treating with several 5 cc portions of the HBr- $\text{Br}_2$  mixture, (l), evaporating to dryness on the steam bath after each treatment. Take up with 2–3 cc of  $\text{HNO}_3$ , evaporate to dryness to expel Br and take up with hot  $\text{H}_2\text{O}$ . Filter, adjust acidity to 1% with  $\text{HNO}_3$  and proceed as directed in 19 and 20.

(l) *Hydrobromic acid-bromine mixture*.—To 250 cc of 40% redistilled HBr add 35 cc of redistilled liquid Br.

(m) *Sodium polysulfide*.—Dissolve 480 g of  $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$  and 40 g of NaOH in

H<sub>2</sub>O, add 16 g of powdered sulfur, shake until the sulfur dissolves, filter, and dilute to 1 liter.

(n) *Hydrochloric acid-citric acid*.—Add a quantity of Reagent (d) equivalent to 50 g of citric acid to 50 cc of HCl and dilute to 250 cc.

(o) *Sodium oleate soln.*—10%. To 45 cc of 30% NaOH and 400 cc of H<sub>2</sub>O in a 1.5 liter beaker, add slowly while heating and stirring 90 g (by difference from a separatory funnel) of oleic acid. Heat the mixture on the steam bath until the soap is entirely dissolved. (A small flocculent precipitate of impurities may remain.) Cool, dilute to 1 liter, mix, and filter.

(p) *Ammonia-cyanide-citric soln.*—Dissolve 10 g of KCN or NaCN and 10 g of citric acid in 500 cc of NH<sub>4</sub>OH (sp. gr. 0.90) and dilute to 1 liter. Preserve in a dispensing apparatus that will minimize loss of NH<sub>3</sub> by volatilization.

## 14

## PREPARATION OF SAMPLE (ASHING)

The quantity of material taken for a sample depends upon the amount available and the expected Pb content, and whether the Pb is to be determined as directed in 19 and 20 or 22 and 23. The Pb ranges for which these procedures are most applicable are given in Fig. 35. In general, weigh a representative sample of 5–200 g, depending upon conditions, into a porcelain dish or casserole of convenient size. Dry wet samples on the steam bath or in a hot water oven. Add 2–5 cc of the “ash-aid” soln, 13(c), to products difficult to ash (meats), or to furnish ash bulk to low ash products (candies and jellies low in fruit content); mix well, and dry. Char gelatin, carbohydrate foods, such as jam and other products that have a tendency to swell excessively, by carefully heating over a burner. (Swelling can be controlled by playing a small flame from a glass jet over the surface of the material in the dish, but do not use a metallic burner because of possible metallic contamination.) Do not allow the material to ignite. Milk, candies, etc., may be charred by adding the sample a little at a time to a casserole heated over a burner or hot plate. When the samples are dry or charred, place them in a temp.-controlled muffle and raise the temp. slowly to 500° without ignition. If the sample contains fat, “smoke” it away by heating a sufficient length of time at about 350°. Cover the floor of the muffle with a piece of asbestos board or silica plate so that the sample receives most of its heat by radiation from the sides and roof and not by conduction from the hotter floor of the muffle.

If the muffle is provided with an automatic control, conduct the ashing overnight at not over 500°. If the sample is not completely ashed the next morning or if day-time ashings at 500° are not proceeding satisfactorily, remove the casserole, cool, and moisten the char with 2–5 cc of the ash-aid. Dry the contents of the casserole past danger of spattering (no free liquid) and replace it in the muffle. If ashing is not complete or proceeding rapidly after 30 min., remove the casserole, cool, and cautiously add 2–3 cc of HNO<sub>3</sub>. Dry, place in the muffle, and continue the ashing until practically carbon-free. Avoid the excessive use of ash-aid and particularly HNO<sub>3</sub>, if the sample still contains much intermixed carbon, because local overheating or deflagration may result, especially if much potassium is present in the ash.

When a clean ash is obtained, cool, cover the casserole with a watch-glass, and add cautiously 15–20 cc of HCl. Rinse down the watch-glass with H<sub>2</sub>O and heat on the steam bath. If a clear soln is not obtained, evaporate again to dryness and repeat the addition of HCl. (Phosphates are sometimes difficult to dissolve entirely, but the addition of 10–15 cc of 60% HClO<sub>4</sub> (double distilled preferred) and



evaporation to fumes on the hot plate are usually effective in obtaining a clear soln. aside from silica or particles of carbon. If  $\text{HClO}_4$  is used, considerable  $\text{H}_2\text{O}$  (200 cc) may be necessary to completely dissolve potassium perchlorate later, especially if KCN instead of NaCN is used in the dithizone extraction of Pb, 16.)

Dilute with  $\text{H}_2\text{O}$  and filter the soln when necessary with suction thru a fritted glass filter (Jena 11G4 is preferable). Catch the filtrate in a 500 cc glass-stoppered Erlenmeyer flask under a bell-jar. Leach insoluble material on the filter successively with a few cc of the hot  $\text{HCl}$ , the hot  $\text{HCl}$ -citric acid soln, and the hot ammonium acetate. Finally add to the casserole a few pellets (2-3 g) of NaOH and dissolve in a few cc of hot  $\text{H}_2\text{O}$ . Tilt the dish so that the sirupy soln wets completely that portion of the interior originally occupied by the sample, then heat on the steam bath until the interior is nearly dry. Take up the residue with  $\text{H}_2\text{O}$  and add directly to the filtrate so as not to redissolve any silica on the filter. Finally rinse the dish with a few cc of hot  $\text{HCl}$  followed by hot  $\text{H}_2\text{O}$ . (This treatment with NaOH dissolves any Pb baked on the dish during ashing; it is particularly necessary when (1) the ashing is of long duration, (2) no ash-aid has been used, or (3) the natural ash is low. Porcelain retains Pb to a lesser extent than does silica.)

## 15

## ISOLATION OF LEAD

At this point proceed as directed under the dithizone extraction of the alkaline soln of the ash, 16, or under the isolation by means of a sulfide precipitation, 17. Procedure 16, while rapid and convenient, is limited to those materials which, with the aid of citric acid, will yield the clear ammoniacal soln demanded for quantitative extraction of Pb with dithizone. Lead is readily occluded by many alkaline precipitates (Mg and Ca phosphates, Al and ferric hydroxides and silicates). Many food materials may be handled in this way as the naturally occurring amounts of these substances are not excessive. However, some materials contain more of these substances than can be kept in soln under alkaline conditions with any reasonable amount of citric acid. In these cases proceed as directed under 17. The difficulty of ammoniacal precipitation may sometimes be overcome by limiting the sample size in those cases where sampling is no problem.

## 16

## DITHIZONE EXTRACTION

(Applicable to most carbohydrate and cereal foods, fruit and fruit products, milk, fresh vegetables, plant materials, etc.)

Transfer the ash soln to a 300 cc short-stemmed separatory funnel and add citric acid reagent, 13(d), equivalent to 10 g of citric acid. Make slightly alkaline to litmus with ammonia, keeping the soln cool, and allow to stand 2-5 min. If a precipitate forms, redissolve with  $\text{HCl}$  and isolate the Pb as directed under 17. If no precipitate forms, add 5 cc of 10% KCN or NaCN soln (more may be necessary if large quantities of Zn, Cu, Cd, etc., are present) and check the pH of the soln by adding a drop of thymol blue and observing the color of the drop. (The pH should be 8.5 or above, blue-green to blue with thymol blue. If the ash was highly colored with Fe, the pH of the soln should be kept comparatively low, because a pII of 10 or above in the presence of Fe may cause oxidation of the dithizone.) Immediately extract with 20 cc portions of the dithizone reagent, using the weaker solns unless exceptionally large quantities of Pb are present. Shake 10-15 seconds, allow the layers to separate, and note the color of the  $\text{CHCl}_3$  phase. (The Pb dithizone complex is red, but the color may be masked by excess green dithizone, giving intermediate hues of purple and crimson. The color of the  $\text{CHCl}_3$  extract gives the first indication of the amount of Pb present, and the progress of the extraction can be followed by noting the color of successive extracts.)

(a) *If the Pb is to be determined electrolytically* ( $\text{Pb} > 0.05 \text{ mg}$ ), draw off the  $\text{CHCl}_3$  layer into a 125 cc short-stemmed separatory funnel containing 25–30 cc of  $\text{H}_2\text{O}$  made ammoniacal with *one drop* of ammonia (sp. gr. 0.90). Continue the extraction until two successive extracts with small portions of the weaker dithizone solns show the negative green (not bluish or purple) color, combining the extracts in the smaller separatory funnel. Shake, allow the layers to separate, draw the  $\text{CHCl}_3$  fraction into another small separatory funnel, and repeat the washing process as before. Draw off the  $\text{CHCl}_3$  fraction as cleanly as possible into a 100 or 150 cc beaker, and pass a small portion of the dilute dithizone soln thru the funnels in succession so as to wash out small portions of the extract persisting in the aqueous fraction. Add to the beaker and evaporate the  $\text{CHCl}_3$  with gentle heat on the steam bath. Take up the dry residue with 3–4 cc of  $\text{HNO}_3$ , and heat by swirling over a low flame. Dilute to approximately 25 cc and continue the heating 1–2 min. in order to fume off oxides of N. Add a small piece of litmus paper, neutralize with strong ammonia, dilute nearly to the capacity of the beaker, and add 1 cc of water-white  $\text{HNO}_3$  per 100 cc of soln. Proceed as directed under 19 and 20.

(b) *If the Pb is to be determined by the colorimetric dithizone procedure* ( $\text{Pb} < 0.2 \text{ mg}$ ), do not wash the dithizone extracts with the dilute ammonia, but run directly into a smaller separatory funnel containing 25 cc of the 1%  $\text{HNO}_3$ , 13(b). When extraction is complete, shake the combined extracts in the smaller separatory funnel and draw off the green dithizone into another separatory funnel containing a further 25 cc portion of 1%  $\text{HNO}_3$ . Shake, allow the layers to separate, and discard the  $\text{CHCl}_3$  fraction. Filter the acid extracts containing Pb in succession thru a small pledget of wet cotton inserted in the stem of a small funnel, into a 50 cc flask or glass-stoppered cylinder, using the second acid extract to wash out the funnel in which the first acid extraction was made. (This procedure removes  $\text{CHCl}_3$  globules.) Make up any slight deficiency in volume with the 1%  $\text{HNO}_3$  and mix. Proceed as directed under 22 and 23. (This method of final isolation in 1%  $\text{HNO}_3$  is unsuited to the electrolytic determination, as small amounts of  $\text{CHCl}_3$  dissolved in the acid cause chlorides to develop under the conditions of the electrolysis.)

## 17

*Sulfide Separation*

(Applicable to all products and usually necessary in the case of cacao products, tea, sardines, and all food products containing a high proportion of alkaline earth phosphates, especially those of Mg, which promote the formation of precipitates in ammoniacal citrate solns.)

Cool the acid soln of the ash, add the citric acid reagent, 13(d), equivalent to 10 g of citric acid, and adjust to a pH of 3.0–3.4 (bromophenol blue) with ammonia. If enough Fe is present to color the soln strongly, make the final adjustment with the help of a spot plate. (Precipitated phosphates from local action of  $\text{NH}_4\text{OH}$  may usually be redissolved by shaking and cooling.) If the amount of Pb is small, add 5–10 mg of pure  $\text{CuSO}_4$  to the soln to act as a coprecipitant. Precipitate the sulfide by passing in  $\text{H}_2\text{S}$  until the soln is saturated (3–5 min.). Immediately filter with suction into a flask in a bell jar (fritted glass filter, Jena 11G4 or equivalent, is preferable).

(a) *If the Pb is to be determined electrolytically* ( $\text{Pb} > 0.05 \text{ mg}$ ), wash the flask and precipitate with a few small portions of 3%  $\text{Na}_2\text{SO}_4$  adjusted to pH 3.0–3.4 and saturated with the  $\text{H}_2\text{S}$  reagent. If a clean sulfide precipitate has been obtained, dissolve the sulfides with 5 cc of hot  $\text{HNO}_3$ , wetting all portions of the filter; allow to stand a few minutes and draw thru into the flask in which the sulfide precipita-

tion was made. Wash the filter with several portions of hot  $H_2O$ , stopper the flask, shake, and boil for a few minutes to remove traces of  $H_2S$ . Cool, adjust the acidity to 1% with  $HNO_3$  in 100–125 cc volume and proceed as directed in 19 and 20. If there is a possibility of the sulfide precipitate being contaminated with  $Cl$ ,  $As_2O_3$ ,  $P_2O_5$ , or  $Hg$  in excess of the quantities shown in Fig. 35, or with  $Sb_2S_3$ , dissolve as directed above with  $HNO_3$  (without the previous washing with  $Na_2SO_4$  soln), wash the filter with hot  $H_2O$ , and boil the soln as before. Transfer to a 200 cc separatory funnel, add the citric acid reagent equivalent to 5 g of citric acid, make ammoniacal and extract with dithizone soln as directed under 16, 16(a), 19, and 20.

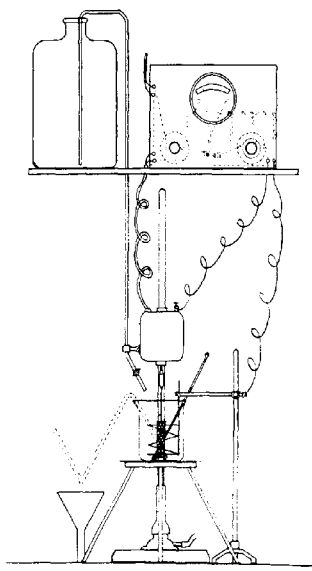


FIG. 36.—ELECTROLYTIC APPARATUS

(b) If the  $Pb$  is to be determined by the colorimetric dithizone procedure ( $Pb < 0.2$  mg), dissolve the sulfides, without previous washing, with 5 cc of hot  $HNO_3$ , drawing the soln thru into the original flask; wash with hot  $H_2O$ , stopper, shake, and boil to remove  $H_2S$ . Transfer to a 200 cc separatory funnel, add the citric acid reagent equivalent to 5 g of citric acid, make ammoniacal, and extract as directed under 16, 16(b), 22, and 23.

#### *Electrolytic Determination*

18

#### *Apparatus*

A diagram of the apparatus used is shown in Fig. 36. Four dry cells in series constitute a convenient source of current. The meter (0–500 milliamperes), switch, fuse, rheostat (60 ohm radio type), and variable resistance for the control of the motor

speed (25–500 ohm,  $\frac{1}{2}$  amp. capacity) may be conveniently mounted upon a panel. The motor for rotating the anode (1/20 H.P., 110 v. universal) is equipped with a chuck and binding post. The rate of rotation should be sufficient to produce efficient circulation and may vary from 400–800 r.p.m. The electrodes consist of a 45-mesh, sand-blasted, Pt gauze; cylindrical anode  $1'' \times 5/16''$  and 4' overall length, and a cathode of 18-gage Pt wire wound in spiral form. (For larger amounts of Pb (over 5 mg) a cylindrical anode  $2'' \times \frac{1}{2}''$ , is convenient.)

## 19

*Electrolysis*

Immediately before electrolyzing bring the anode to red heat in the oxidizing flame of a burner. (A somewhat variable titration blank is obtained if the anode is not heated just before the determination, due possibly to a film of oxygen adsorbed on the anode and activated during electrolysis. Heating reduces and renders constant this "oxygen blank." With the small anode it will be 0.07–0.1 cc of 0.001 *N* thiosulfate and with the larger electrode proportionately larger. The blank for a particular anode should be determined from the average of a series of determinations conducted on pure reagents.)

In all determinations the sample at this point is contained in a volume of 100–125 cc of 1%  $\text{HNO}_3$  (with the large anode a volume of 200 cc is convenient). Place the beaker (100–150 cc for the small and 250 cc for the large anode) in position, making sure the electrodes are well covered with soln, and start the motor. Heat to 60–70°, and then add about 100 mg of  $\text{K}_2\text{Cr}_2\text{O}_7$  to keep the soln in an oxidized state and repress the formation of nitrites, especially when organic matter is present. Start the current and electrolyze with about 75 milliamperes for 20 min. at 70–80°. (Use 100–150 milliamperes for the larger anode.) Remove the flame, insert the siphon in the beaker, and start a stream of distilled  $\text{H}_2\text{O}$  playing directly on the anode. Start the siphon, taking care to keep the level of the liquid above the deposit. A convenient siphon can also be made by connecting an inverted V-shaped tube to an ordinary water-pump. The acid is entirely removed when the current falls to zero. Turn off the motor, electrolysis current, and rinse water; remove the anode from the chuck and give it a final rinse with distilled  $\text{H}_2\text{O}$ .

## 20

*Titration of  $\text{PbO}_2$* 

Dissolve the deposit in 4–5 cc of the "stripping" reagent, 13(f) + 1 cc of the KI reagent contained in a flat-bottomed vial of such size that the soln just covers the anode. Add a few drops of the starch soln, 13(h), and titrate the liberated I with 0.001 *N* thiosulfate, 13(i), in the vial, using the anode as a stirrer and sighting down thru the vial, as thru a miniature Nessler tube, to detect the delicate end-point. (If the quantity of Pb is seen to be large (1–5 mg), use 0.005 *N* thiosulfate and double the amount of the reagents 13(f) and (g). With the 2 in. anode still larger amounts may be used.) No yellow insoluble  $\text{PbI}_2$  should form as the deposit is "stripped"; if it does add more of the Na acetate. (The deposit should dissolve completely and almost immediately.) To determine the amount of Pb, subtract the anode and reagent blanks from the total titer and multiply by the factor of the thiosulfate, 13(i).  $\text{PbO}_2 + 4\text{HI} = \text{I}_2 + \text{PbI}_2 + 2\text{H}_2\text{O}$ . The absence of interfering Bi may be assured by applying test 26(d).

## 21

COLORIMETRIC DITHIZONE DETERMINATION<sup>1</sup>

The limiting factor in the determination of minute quantities of Pb by the colorimetric dithizone procedure is probably the size of the reagent blank. The importance

of careful blank determinations must be especially stressed when quantities of Pb of the order of 1  $\gamma$  ( $1\gamma=0.001$  mg) are being determined. With special care in purification of reagents and by the use of carefully cleaned Pyrex ware, including separatory funnels, it should be possible to reduce the reagent blank to 1  $\gamma$  or possibly below. Owing to Pb-bearing dust, vapors, etc., it is necessary to expose the blank determination in the muffle or on the steam bath for the same length of time as the sample is exposed, and to use exactly the same amounts of reagents (even  $H_2O$ ) for the blank and actual determinations.

Pb is extracted from aqueous soln, under standard conditions of volume and pH, with a definite volume of a  $CHCl_3$  soln of dithizone of standard strength. The optimum pH of operation is 9.5-10.0. Dithizone strengths are so chosen that an excess of dithizone is always present in the reaction mixture. Lead is brought into the  $CHCl_3$  phase in the form of the red complex, and the uncombined green dithizone partitions between the aqueous and  $CHCl_3$  phases and modifies the color of the extract according to the relative amounts of Pb and dithizone. Thus, according to this proportion, a series of colors from red to green may be arranged with intermediate crimsons, purples and blues. The volumes and strengths of the  $CHCl_3$  solns depend upon the Pb range it is desired to cover and are so chosen as to give the same general color progression from red to green for each range. Limiting the range increases accuracy at the expense of flexibility. The colors produced with standard amounts of Pb furnish by comparison the basis for a quantitative estimation. The volumes and concentrations of standard dithizone for various ranges are as follows:

Pb RANGES gamma (0.001 mg)	CONCENTRATION mg/l	VOLUME cc	CELL LENGTH inches
0-5	4	5	2
0-10	4	10	2
0-20	8	10	1
0-50	8	25	1
0-100	10	30	$\frac{1}{2}$
0-200	20	30	$\frac{1}{2}$

## 22

*Simple Color Matching*

Prepare 10 standards covering in equal steps the range in which it is desired to work, as follows: Use a standard Pb soln, 13(a), in 1%  $HNO_3$ , 1 cc of which equals some simple fraction or multiple of 1  $\gamma$  of Pb. Measure the amounts representing the various steps of the range into a series of separatory funnels and add the pure 1%  $HNO_3$  so that the total volume is always 50 cc. (It is best to add the acid first so that the Pb soln is not lost around the stopcock of the separatory funnel.) Add 10 cc of the ammonia-cyanide mixture, 13(j), and mix. The resultant pH will be about 9.7. Immediately add the appropriate volume of standard dithizone, which depends upon the range to be covered (see table), and shake for 1 min. Draw off the lower layers into a series of tubes or vials and arrange in order. For the lower ranges, i.e., up to 20  $\gamma$  of Pb, matching is best done by viewing longitudinally in small flat-bottomed vials about 3 in. in length. For the higher ranges, i.e. 0-50  $\gamma$  and above, depth of column must be reduced, and matching is conveniently done by viewing transversely in Nessler tubes of matched diameter. This is because even pure dithizone solns appear red by transmitted light if the concentration or depth of column is increased beyond a certain point. If standards are kept covered when not in use they should last at least one day.

For the determination, place an aliquot part, or the entire amount, of the 50 cc of 1%  $\text{HNO}_3$  in which the Pb has been isolated, 16(b) and 17(b), in a separatory funnel, and if an aliquot is taken, make to 50 cc with the 1%  $\text{HNO}_3$ . Add 10 cc of the ammonia-cyanide mixture, 13(j), and mix. Immediately develop the color by shaking 1 min. with the proper amount of the standard dithizone. Draw off the lower layer into a tube or vial similar to those used with the standards and compare. If the range is exceeded, repeat with a smaller aliquot, or re-extract with excess dithizone before draining from the funnel, isolate once more in 50 cc of the  $\text{HNO}_3$  reagent, and compare with standards covering a higher range. Interpolation between steps of the various ranges should be easily made. If an aliquot of the 50 cc of the 1%  $\text{HNO}_3$  in which the Pb had been isolated is taken, subtract only a corresponding amount of the total reagent blank from the amount of Pb found.

## 23

*Photometric Methods*

Transmission spectra of the two components in the dithizone extract, viz., the Pb dithizone complex and the free dithizone, show a marked difference in their ability to absorb light of wave length  $510 \text{ m}\mu$ , the red Pb complex absorbing strongly and the free green dithizone transmitting freely. Thus, when the absorption of light of this wave length by the individuals of a standard color series, measured thru a suitable cell-length, is determined photometrically, a practically linear relation is observed between amounts of Pb and absorption coefficient ( $-\log$  transmittancy). In making the measurements a spectrophotometer set at this wave length, or a simple photometer equipped with a blue-green filter centered at about this point can be used. The dithizone solns are standardized once only with known amounts of Pb, and the labor of repeated standard preparation is necessary only when changes caused by evaporation or oxidation occur.

Standardize dithizone solns as follows: Using the appropriate volumes and concentrations specified for the various ranges (see above), prepare standard colors as in the visual color-matching procedure, saturating the standard Pb and the 1%  $\text{HNO}_3$  solns with clear  $\text{CHCl}_3$  before use, and thereby eliminating differences in volume of extract between standards and unknowns. (It is unnecessary to prepare the full 10 steps of the range, and the number of standards may be limited to 5 or 6.) Develop the colors by shaking 1 min., allow to stand a few minutes, and filter the extract thru specially prepared filter papers (9 cm quantitative filters soaked overnight in 1%  $\text{HNO}_3$  and washed with large volumes of  $\text{H}_2\text{O}$  on a Biehner funnel to remove the slight trace of acid and/or Pb usually present on even the best grades of filter paper. Fitting a 9 cm filter directly into the mouth of a 50 cc low-form Pyrex beaker eliminates the use of a funnel in the filtering operation). Fill a cell of proper length with the filtered extract for the various Pb ranges, as indicated in the table given previously, using the specified volume and strength of standard dithizone solns. Use cells of all glass (preferably Pyrex) construction with plane parallel fused ends.

Determine *absorption coefficients* for the various steps of the range and plot against the quantity of Pb to obtain a standardization curve for the particular lot of dithizone. Preferably calculate the slope of the line connecting the standard points and the intercept of the line on the Pb axis, making the calculation as follows: Take the equation of the line connecting the standard points as  $X = a + bY$ , and let  $X = \gamma$  of Pb and  $Y = \text{absorption coefficient}$ ;  $a$  then represents the intercept on the Pb axis (in this case a negative value) and  $b$  represents the tangent or slope of

the line. Calculate  $a$  and  $b$  from the following formula, where  $n$  = No. of observations, including that for 0 lead, and  $\Sigma$  represents merely "the sum."

$$b = \frac{\Sigma XY - \frac{\Sigma X \Sigma Y}{n}}{\Sigma Y^2 - \frac{\Sigma Y \Sigma Y}{n}}, \text{ and } a = \frac{\Sigma X}{n} - b \frac{\Sigma Y}{n}.$$

Then the procedure for determining the Pb content of an unknown falling within the range is to determine the value of the absorption coefficient, using the standard dithizone and the same cell with which the standard readings were made, and calculate the Pb from the equation,  $X = a + bY$ , using the values of  $a$  and  $b$  as determined above. If protected from evaporation and direct sunlight the standard factors of dithizone solns should not change appreciably for at least a month.

For the actual determination proceed as directed in 22, except to filter the extract before photometric measurement thru the prepared filter papers. Determine the absorption coefficient, using the standardized dithizone with the same cell used in making the standard curve, and read the amount of Pb from this standard curve or calculate from the factor of the dithizone soln. If the range is exceeded, repeat with a smaller aliquot, or re-extract and repeat with dithizone standardized to cover a higher range. If an aliquot of the 50 cc of 1%  $\text{HNO}_3$  in which the Pb has been isolated is taken, subtract only a corresponding amount of the total reagent blank from the amount of Pb found.

## 24

## INTERFERENCES

(a) *In the electrolytic method.*—If present in excessive quantities in the final determination, Cl,  $\text{P}_2\text{O}_5$ , As, Se, Te, Hg, and Bi (>5 mg) will prevent the complete deposition of Pb, and Bi (<2 mg), Sn, Sb, Mn, and Ag will contaminate the deposit. Certain reducing agents, such as nitrites, likewise prevent complete deposition of the Pb. The general method leading up to the final determination of Pb by the electrolytic procedure has been so formulated that all interferences except those of Sn and Bi are eliminated. As much as 3 mg of Cl, 20 mg of  $\text{As}_2\text{O}_3$ , 30 mg of  $\text{P}_2\text{O}_5$ , and 50 mg of Hg will not interfere in the final electrolysis, and if there is suspicion that greater quantities are present in the sulfide mixture, 17, they can be eliminated by a dithizone extraction. Tin becomes a problem in the analysis of canned foods, and in amounts above 150 p.p.m. it will usually be found in the ash as insoluble  $\text{SnO}_2$ . It appears as a milky white suspension in the ash soln.

(b) *In the colorimetric dithizone method.*—These are limited by the use of KCN to stannous Sn, Bi, and Tl. The rarity of Tl makes its interference unlikely in ordinary work, and no method of removal is given. Procedures are given for the removal of Sn and Bi, 25 and 26. Dithizone itself is destroyed by strong oxidizing agents, and the presence of free halogens and large amounts of ferric Fe may prove troublesome in the dithizone extraction.

## 25

## REMOVAL OF TIN

Proceed as directed for volatilization as  $\text{SnBr}_4$ , (a) and (b), or for leaching the mixed sulfides with warm sodium polysulfide, (c), when the sulfide method of isolation, 17, has been used.

The volatilization procedure may be applied directly upon the acid soln of the ash as directed in (a) or after isolation of the Pb and any contaminating Sn according to the dithizone isolation or sulfide method, (b). Procedure (a) may not remove all traces of Sn when large amounts of other ash salts are present, as the  $\text{SnBr}_4$  may be mechanically entrapped. However, the amount will be reduced to below that necessary to interfere with the final electrolytic determination of Pb. Accordingly, (a) is recommended as a preliminary step to a dithizone isolation of lead, 16, preparatory to an electrolytic determination, because the small amounts of Sn that may escape volatilization will be oxidized to the stannic form, which does not react with dithizone.

Volatilization of Sn is complete when the metals have been previously isolated, (b), and complete elimination of Sn is necessary if the more delicate dithizone method, 21-23, of the final determination ( $\text{Pb} < 0.05 \text{ mg}$ ) is to be applied. Volatilization may also be applied when only small amounts of contaminating Sn are present.

Procedure (c) is recommended for routine work by the electrolytic method on account of its speed, but as all traces of Sn may not be removed by this procedure, complete elimination by procedure (b) is necessary if very small quantities of Pb are being determined by the colorimetric dithizone method.

(a) *Volatilization as  $\text{SnBr}_4$  from the acid soln of the ash.*—After a practically carbon-free ash has been obtained, 14, add 15–20 cc of 40% redistilled HBr. If nitrates have been used as ash aids, cover the casserole with a watch-glass and heat on the steam bath until Br evolution diminishes, then rinse off the watch-glass with  $\text{H}_2\text{O}$  and bring to a boil to complete expulsion of Br. (This process destroys undecomposed nitrates.) Add more HBr, if necessary, to dissolve the ash and examine the solns for clearness. If there is an insoluble residue of  $\text{SnO}_2$ , add 50–100 mg of pure Sn, 13(k), to the simmering HBr soln of the ash and allow it to dissolve. (Metallic Sn seems to be the best agent to bring ignited  $\text{SnO}_2$  into soln. To be effective the ash soln must be in the reduced state. Ferric oxide sometimes becomes “noble” during ashing and dissolves with difficulty, but treatment with metallic Sn also brings it into soln. Treatment with Sn will be necessary only with the contents of badly corroded cans.) When soln of the ash is free from milkiness due to  $\text{SnO}_2$ , add 20 cc of 60%  $\text{HClO}_4$  (double distilled preferred), oxidize the mixture with a few cc of the HBr– $\text{Br}_2$  mixture, 13(l), and then add a further 15 cc of the reagent, portionwise, while the soln is evaporated to incipient fumes of  $\text{HClO}_4$  (approximately  $150^\circ$ ) on a hot plate. Repeat with another 10 cc portion of the HBr– $\text{Br}_2$  mixture if more than 100 mg of tin has been used to dissolve the ash. (Hot  $\text{HClO}_4$  helps keep the ash salts in soln and with Br holds the Sn in the volatile  $\text{SnBr}_4$  combination.) When the Sn, HBr, and Br have been completely volatilized, cool, and take up with hot  $\text{H}_2\text{O}$  (200 cc may be necessary if much  $\text{KClO}_4$  is present). Filter off any small quantities of dehydrated silica, extract the residue twice with 5 cc of the hot HCl–citric acid reagent, 13(n), and hot  $\text{H}_2\text{O}$ , treat the dish with NaOH as directed in 14, and isolate the Pb by dithizone extraction as directed under 16, or by sulfide separation, 17, finally determining Pb as directed under 16(a), 17(a), 19, and 20.

(b) *Volatilization as  $\text{SnBr}_4$  after isolation of Pb.*—(Recommended for the complete elimination of Sn remaining from volatilization as  $\text{SnBr}_4$ , (a), or present as traces in the original sample; necessary when very small quantities of Pb are being determined colorimetrically.)

Isolate the Pb, along with the small amounts of interfering Sn, by means of a dithizone extraction, 16, or by a sulfide precipitation, 17. If a dithizone extraction



is used, extract the metals from the dithizone extract with one 50 cc portion of HCl (1+10). Rinse the acid soln into a casserole and evaporate to dryness on the steam bath. Treat with 10-15 cc of the HBr-Br<sub>2</sub> mixture, 13(d), added a few cc at a time, and evaporate to dryness after each addition. Take up the Sn-free residue with a few cc of HNO<sub>3</sub> and heat to expel Br. When the soln is water-white transfer to a separatory funnel with 75-100 cc of H<sub>2</sub>O and isolate the Pb as directed under 16, proceeding, 16(b), to the colorimetric dithizone determination, 22 and 23. If the quantity of Pb is over 0.05 mg, the Pb may be determined electrolytically, 16(a), 19, and 20.

If a sulfide separation is used, 17, dissolve the mixed sulfides from the filter with 5 cc of hot HNO<sub>3</sub>, rinse thru into the original flask with hot H<sub>2</sub>O, stopper, shake to dissolve any remaining sulfides, and then transfer the soln to a casserole. Evaporate to dryness and volatilize Sn with HBr-Br<sub>2</sub> as directed under (b).

(c) *With sodium polysulfide*.—(Recommended for routine work on canned foods by the electrolytic method when Pb > 0.05 mg.)

Isolate the Pb by means of a sulfide precipitation, 17, filter, and wash the flask and filter with 3-6 portions of about 5 cc each of the warm sodium polysulfide, 13(m). (Sn, As, and Sb sulfides are dissolved; CuS may be partially dissolved and reprecipitated in the filtrate.) Wash the flask and residual sulfides several times with 3% Na<sub>2</sub>SO<sub>4</sub> adjusted to pH 3.0-3.4 and saturated with H<sub>2</sub>S, and proceed as directed under 17(a), beginning "dissolve the sulfides with 5 cc of hot HNO<sub>3</sub>," and continuing directly to the electrolytic determination, 19 and 20. (When the ash contains much Sn, as when metallic Sn has been added to dissolve insoluble metallic oxides, the sulfide precipitate will be so bulky as to be difficult to handle, and it will be necessary to use the volatilization procedure, (a).)

## 26

## DETECTION AND REMOVAL OF BISMUTH

(a) *From the acid soln of the ash*.—To the acid soln of the ash, 14, before or after volatilization of SnBr<sub>4</sub>, 25(a), in a separatory funnel, add 2 cc of 10% KI soln. Shake with 35-50 cc of ethyl acetate washed previously with 10 cc of H<sub>2</sub>O, and allow the layers to separate. A colorless layer confirms the absence of Bi. If the upper layer is colored yellow to orange-red, draw it off into a 100 cc beaker and evaporate to dryness on the steam bath. Moisten the interior of the beaker with 3-4 drops of HNO<sub>3</sub> and again evaporate to dryness to volatilize any I. Complete the evaporation by waving the beaker over a low flame. Add 1-2 drops of HNO<sub>3</sub> and about 1 cc of H<sub>2</sub>O and heat to bring any residue into soln; if colored yellow, repeat the evaporation with HNO<sub>3</sub> and add, if necessary, a small crystal of KClO<sub>3</sub>. When a colorless soln containing 1-2 drops of HNO<sub>3</sub> in 1 cc of H<sub>2</sub>O is obtained, pour it into a small test tube. Add an equal volume of a 10% soln of thiourea, rinsing out the beaker with the same soln before adding it to the test tube. A yellow color indicates Bi. (As little as 0.003-0.005 mg of Bi in the original ash soln will show a yellow color by comparison against a negative test. Larger quantities yield decidedly yellow colors.) If the Bi test is positive, make 3 or more extractions with washed ethyl acetate (35-50 cc) according to the yellow shade of the Bi test. Remove residual ethyl acetate by extracting twice, each time using 10 cc of CHCl<sub>3</sub>. Then determine the Pb electrolytically, 19-20, either by dithizone extraction, 16, 16(a), or after sulfide separation, 17. (For assurance that Bi has been sufficiently removed, apply test (d).) If a colorimetric determination is necessary (Pb < 0.05 mg), extract the Pb and any residual Bi from the soln after the ethyl acetate ex-

traction with dithizone as directed under 16, and proceed as directed under (b).

(b) *By dithizone at pH 2.0 after preliminary dithizone extraction at pH 8-11.*—(This procedure completely removes Bi and stannous Sn.)

Extract the metals from the  $\text{CHCl}_3$  dithizone extract with 50 cc of 1%  $\text{HNO}_3$  as directed in 16(b). Adjust the acid extract to pH 2.0 (metacresol purple indicator) with 5%  $\text{NH}_4\text{OH}$  and shake vigorously for about 1 min. with 10 cc of a  $\text{CHCl}_3$  soln of dithizone containing 200-250 mg per liter. Allow the layers to separate, and if the  $\text{CHCl}_3$  extract is red, draw it off and extract with another 10 cc portion of the dithizone soln. If shades of green or purple are visible, indicating an excess of dithizone, draw off the  $\text{CHCl}_3$  extract and extract the aqueous phase once more with 5 cc of the dithizone soln (the shaking should be prolonged, 3-5 min., to insure the complete extraction of Bi). Continue the extractions until the dithizone extract remains a pure green. Adjust the pH of the aqueous soln to 8.5 with  $\text{NH}_4\text{OH}$ , add KCN, and extract with dithizone as directed in 16. Continue the colorimetric determination of Pb as directed in 16(b), 22, and 23, or electrolytically, 16(a), 19, and 20 when the Pb > 0.05 mg.

(c) *From acid soln of sulfides.*—Isolate the Pb by means of a sulfide precipitation, 17, and dissolve the mixed sulfides with hot  $\text{HNO}_3$ . Wash, and then evaporate the soln to dryness in a porcelain dish and treat with small portions of  $\text{HBr}-\text{Br}_2$  mixture, 13(1), on the steam bath to volatilize possible Sn and to convert the metals to the bromides. (If a Bi compound is being analyzed 1 g may be dissolved in  $\text{HBr}$  directly.) Evaporate to dryness and place in a temp.-controlled muffle at 300-325° for 5 min. As and Sb bromides will be volatilized, and the Bi bromide will volatilize in the form of dense orange fumes. At the end of 5 min., or when fumes are no longer evolved, remove the dish, cool, and take up any insoluble residue with hot  $\text{HNO}_3$ . Evaporate to dryness and again treat with small portions of the  $\text{HBr}-\text{Br}_2$ , evaporate to dryness, and heat for an additional 5 min. period at 300-325°. Remove the dish, cool, and take up the residue in hot  $\text{HNO}_3$ . Isolate the Pb by means of a dithizone extraction, 16, continue as directed under 16(a), and determine Pb electrolytically, 19-20. If the sensitive colorimetric dithizone procedure is to be applied, residual traces of Bi must be eliminated by procedure (b).

(d) *After  $\text{PbO}_2$  titration in electrolytic method.*—Add to the soln from 20 in the titrating vial 0.25 g of solid KI and about 0.5 cc of  $\text{HCl}$ . Shake, and add only sufficient  $\text{Na}_2\text{S}_2\text{O}_3$  soln to discharge any starch iodide color. A pure yellow color shows the presence of the double bismuth iodide. (Under the conditions of the test, there is no interfering Cu, ferric iron, or Sb, and 0.005 mg of Bi will show the yellow color test.) If the test is positive, reject the Pb results and repeat the determination, giving special attention to the removal of the Bi interference.

#### SPECIAL METHODS OF SAMPLE PREPARATION

##### 27

##### *Solution in Acids*

(Applicable to chemicals soluble in  $\text{H}_2\text{O}$  or acid, e.g., phosphates, sulfates, etc., and organic products of the type of tartrates and citrates.)

Dissolve 5-100 g of sample in  $\text{HCl}$  in a 400 cc beaker, gaging the amount of sample according to its nature and the amount of Pb expected. With calcium phosphates use 10-50 g. Dissolve in the smallest practicable volume of soln by warming and adding alternately small quantities of hot  $\text{H}_2\text{O}$  and  $\text{HCl}$ . Filter the soln with suction (fritted glass filter preferred) into a beaker or flask under a bell jar and

leach any residue with 10–25 cc of the hot HCl-citric acid, 13(n), followed by 10–25 cc of hot 40% ammonium acetate. Rinse the beaker and filter with hot H<sub>2</sub>O and cool the soln.

Proceed as directed under 16. If interference by precipitate formation occurs, reacidify and isolate Pb by the sulfide precipitation, 17. If difficulty is experienced in obtaining a clear soln with calcium phosphates at pH 3.0–3.4 (sulfide precipitate may be contaminated with excessive phosphates), redissolve the precipitate, add more citric acid soln, 13(d), readjust the pH, and reprecipitate the sulfides; or make one sulfide precipitation, dissolve the sulfides in hot HNO<sub>3</sub>, boil off H<sub>2</sub>S, and extract the Pb with dithizone, 16. Sometimes difficulty due to precipitate formation in 16 can be obviated by the use of a smaller sample for extraction and colorimetric determination. If Sn or Bi is suspected, remove by the methods described under 25 and 26. Finally determine the isolated Pb electrolytically, 19 and 20, or colorimetrically, 22 and 23.

## 28

*Complete Digestion*

(Applicable to most food or biological products, except fats and oils, oily products, etc.)

Digest a representative sample in a Kjeldahl flask as directed under 3. Distil the arsenic if desired according to the tentative bromate method, 9. If the arsenic is not to be distilled, add 100 cc of H<sub>2</sub>O and sufficient HCl to the residue in the flask to dissolve any calcium sulfate that may be present. Filter on a fritted glass filter, pulverizing any insoluble residue with a flattened stirring rod (anhydrous silica or BaSO<sub>4</sub>). Dissolve any Pb sulfate in the flask and leach the residue on the filter with 10–20 cc of the hot HCl-citric acid soln, 13(n), followed by 10–20 cc of hot 40% ammonium acetate soln. Finally rinse both flask and filter with hot H<sub>2</sub>O. Isolate the Pb by the dithizone, 16, or sulfide precipitation, 17, methods. (In general, the sulfide method is preferable especially when Ba or excessive Cu sulfates are present, as insoluble sulfates readily occlude Pb.) If Bi and Sn are present, remove them as directed in 25 and 26. After isolation determine Pb according to the electrolytic, 19 and 20, or colorimetric method, 22 and 23.

## 29

*Partial Digestion or "Mush"*

(a) *For fruits or vegetables that can be peeled.*—Weigh and peel a representative sample (10–45 apples), including stem and calyx ends with the peels. Transfer the peels to one or more 2000 cc tared beakers, re-weigh, and record the weight of the peel. Add 75–200 cc of HNO<sub>3</sub> to each of the beakers, according to the weight of peel therein, and warm carefully over a gauze or on a steam bath in a fume hood. Stew slowly, while stirring, until initial foaming decreases. Cover the beaker with a watch-glass and continue the heating until a smooth mixture results with little or no stringiness and a greatly diminished evolution of oxides of nitrogen (15–45 min. according to the amount of sample taken. Colloids (pectin) must be sufficiently destroyed to prevent emulsification in subsequent CHCl<sub>3</sub> extractions). Dilute with H<sub>2</sub>O, cool, and transfer the contents of the one or more beakers to a 1000 or 2000 cc volumetric flask. Make to the mark, mix well, and filter. Transfer 100–250 cc of the filtrate to a short-stemmed separatory funnel, add the citric acid, 13(d), equivalent to 5 g of citric acid, make ammoniacal (the soln will darken materially), and proceed with the dithizone extraction as directed in 16. Determine the extracted Pb electrolytically, 16(a), 19, and 20. Correct for the volume occupied by the insoluble matter of the peels by allowing 0.075 cc per g of peel.

(b) *For products other than fruit and vegetable peels.*—(For carbohydrate foods, fresh or canned small fruits or vegetables, jams, apple butter, etc. Sn is often present, while Bi is usually absent.)

Weigh 100–200 g of well-mixed sample into a 1000–2000 cc beaker. To dry samples, add about an equal weight of  $H_2O$ , add 50–150 cc of  $HNO_3$ , and “mush” the mixture as directed in (a). (The duration of the mashing period and the quantity of  $HNO_3$  should be varied according to the product. Colloids, which induce emulsification in the dithizone extraction, should be destroyed so that a clear soln is obtained upon filtration.) Cool, transfer to a 500 cc flask, mix well, and filter. Transfer a 100–250 cc aliquot of the filtrate to a separatory funnel and proceed as directed in (a), concluding with an electrolytic determination. (Interference of Sn is generally negligible.)

#### *Rapid Method Restricted to Apples and Pears*

(Designed for the rapid determination of Pb spray residue on apples and pears. According to convention results should be expressed as grains per lb.; grains lb.  $\times$  143 = p.p.m.)

### 30

#### PREPARATION OF SAMPLE

Weigh 10 or more apples or pears and pull or cut out the stems with a narrow-bladed knife so as to expose the junction of stem and fruit to the action of the solvent, cutting no more of the flesh than necessary. Trim off the sepals (the dried residue of the blossom) so that the solvents have a clear unimpeded entrance to and egress from the calyx cup. Allow the stems and sepals to fall into a large funnel inserted in the neck of a 500 cc volumetric flask. (No harm results if the stems and sepals fall into the flask.) To 25 cc of the 30% NaOH in a 600 cc beaker, add 175 cc of  $H_2O$  and 25 cc of sodium oleate, 13(a), and bring to a gentle boil. Have ready in a wash bottle 250 cc of hot  $HNO_3$  (1+49) or hot HCl (3+97). (HCl is preferred if As is to be determined later,  $HNO_3$  if the Pb is to be determined electrolytically, 33.) Impale each fruit in turn upon a pointed glass rod and immerse in the alkaline soln, with an occasional rotation until the skin begins to check, then remove to the funnel and rinse with a stream of the hot acid, being careful to flush out the stem and calyx ends thoroly, and to allow the rinse acid to flow over the stems and sepals in the funnel. When all the fruit has been thus treated, cool the alkaline soln and add it thru the funnel to the acid soln in the flask. Rinse the beaker and funnel with any remaining acid and with  $H_2O$ , using the entire 250 cc of rinse acid. Cool and make to volume. In a 200 cc Erlenmeyer flask place 10 cc of  $HNO_3$  or HCl to conform to the kind of acid used in rinsing. Thoroly mix the contents of the flask and immediately add 100 cc to the acid in the flask while swirling vigorously. Filter on a rapid filter. If the first portion of the filtrate is cloudy, return it to the filter until a clear filtrate is obtained. Determine Pb as directed in 31 or 32, or electrolytically, in which case use 25 cc of acid and 250 cc of wash soln and proceed as directed under 33.

### 31

#### DETERMINATION WITH NESSLER TUBES

(At least 15 tubes matched for uniformity in color and diameter are necessary.)

(a) *Standards.*—Introduce into each of two 1 liter volumetric flasks 47.5 cc of 30% NaOH. When  $HNO_3$  has been used in rinsing and acidification, 30, add 100 cc of  $HNO_3$  to each flask. When HCl has been used, add 104.6 cc of HCl to each flask.

To one of the flasks add the stock reagent, 13(a), equivalent to 7.27 mg of Pb.

Mark this flask "standard" and the other "blank." Dilute both solns to volume at room temp. and mix. These two solns contain the reagents as they occur in an acidified and filtered sample soln. The "standard" is equivalent in Pb content to an acidified soln from a sample of 1400 g carrying a Pb load (removable by the "stripping" procedure) of 0.020 grain/lb. By a combination of the two solns in suitable proportions the equivalent of any Pb load from 0 to 0.020 grain/lb. may be obtained.

The standard tubes may be made up in intervals corresponding to 0.002 grain/lb., and then interpolation to 0.001 grain/lb. is possible. The following table gives the quantities of "Standard" and "Blank" to be added to the Nessler tubes for each interval. They are conveniently measured into the tube by means of burets.

GRAIN/LB.	STANDARD CC	BLANK CC
0.000	0.0	20.0
0.002	2.0	18.0
0.004	4.0	16.0
0.006	6.0	14.0
0.008	8.0	12.0
0.010	10.0	10.0
0.012	12.0	8.0
0.014	14.0	6.0
0.016	16.0	4.0
0.018	18.0	2.0
0.020	20.0	0.0

Then add to each tube 10 cc of the ammonia citric acid KCN soln, 13(p), followed by 20 cc of standard dithizone soln (20 mg of *purified* dithizone dissolved in 1 liter of  $\text{CHCl}_3$  and preserved in a dispensing apparatus to prevent evaporation). Shake vigorously for 1 min. and allow the layers to separate. The pH of the aqueous phase should be about 9.4 regardless of whether  $\text{HCl}$  or  $\text{HNO}_3$  is used in rinsing and acidification. Stopper each standard tube securely with a new cork stopper. It is unnecessary to make up the entire series of standards if only a portion of the range, for example, 0.010–0.020 grain, lb., is of quantitative interest.

(b) *Comparison*.—Transfer 20 cc portions of the filtrate from 30 to each of three Nessler tubes. First add 10 cc of the ammonia-citric-acid-KCN, 13(p), to each tube; to one tube add 20 cc of standard dithizone soln (see standards) and to the other two tubes 20 cc of clear  $\text{CHCl}_3$ . Shake the tubes vigorously for 1 min. and allow the layers to separate. With a tube of clear  $\text{CHCl}_3$  backing the sample tube (containing the dithizone) and one sample tube containing  $\text{CHCl}_3$  backing each of two standard tubes, compare the color in the lower layer of the sample with that of the standards, looking thru the tubes at right angles to their lengths toward a strong diffused light. (A comparator box similar to the boxes used in colorimetric pH measurements but of larger size will be found convenient.) When working with apple "strip solns," a slight turbidity is produced in the sample tube, which slightly changes the color observed. To compensate for this effect, introduce the same turbidity in the field of view of the standard tubes made up exactly as in the sample, except that  $\text{CHCl}_3$  is substituted for the dithizone soln.

If the range is exceeded, i.e., if the color produced by the sample is redder than the 0.020 grain standard, repeat with a smaller aliquot of the filtrate, making up to 20 cc with the "blank" soln. If, for example, a 10 cc aliquot is taken, the indicated reading must be doubled. After a match has been obtained, calculate the result to the basis of a 20 cc aliquot and a 1400 g sample.

## 32

## DETERMINATION WITH PHOTOMETER

This procedure lends itself readily to photometric methods of measuring the "mixed color" (see 23). Changes in 31 are introduced here to prevent the formation of colors too dense for measurement. Standards contain half the amount of Pb, and the concentration of the dithizone soln is also halved. Use 10 cc instead of 20 cc aliquots of the acidified wash soln, 30.

(a) *Standards*.—Measure the following proportions of "standard" and "blank" solns, 31, into separatory funnels:

Grain/lb.	0	0.005	0.010	0.015	0.020
Standard cc	0	2.5	5.0	7.5	10
Blank cc	20	17.5	15.0	12.5	10

Add 10 cc of the ammonia-citric acid-KCN soln, 13(p), and immediately develop the colors by shaking 1 min. with 20 cc of pure dithizone soln of 10–12 mg/liter strength. Allow to stand a few minutes to cool, filter the  $\text{CHCl}_3$  layers thru specially washed filter papers, 23, and fill into a one-half inch cell. Determine absorption coefficients and plot against grain/lb. of Pb to obtain a standard curve.

(b) *Comparison*.—Place an appropriate sized aliquot of the acidified strip soln in a separatory funnel and make up to 20 cc with the "blank" soln. Add 10 cc of the ammonia reagent, 13(p), and shake out with 20 cc of the standard dithizone soln (10–12 mg/liter). Allow to stand a few minutes to cool (considerable heat is developed in the neutralization of the acid), filter, and read as directed above. Determine the quantity of Pb from the standard curve as prepared in (a) and calculate to the basis of a 10 cc aliquot and 1400 g sample.

## 33

## ELECTROLYTIC DETERMINATION OF LEAD IN APPLE FILTRATE

The principal interferences are chlorides and Mn. Bi and Sn are not expected in spray residue. Chlorides can be avoided by using  $\text{HNO}_3$  for rinsing and acidification. Test for Mn in 10 cc of the acid filtrate obtained, 30, as follows: Add 5 cc of 85% sirupy  $\text{H}_3\text{PO}_4$  and about 0.2 g of potassium periodate and boil gently. Evaporate to a sirupy consistency, adding a little more periodate, if necessary, to decompose organic matter. Cool, and dilute with a few cc of  $\text{H}_2\text{O}$ . The pink color of permanganate ion appears if as little as 0.001 mg of Mn is present. If HCl has been used in rinsing and acidification, boil off the bulk of the HCl with the sirupy  $\text{H}_3\text{PO}_4$  before adding the periodate. If Mn is absent, neutralize 200 cc of the acid filtrate (HCl free) in a 250 cc beaker with  $\text{NH}_4\text{OH}$ , add 2 cc of fresh  $\text{HNO}_3$ , and electrolyze as directed in 19, using the 2 in. electrode and 0.3–0.4 g of  $\text{K}_2\text{Cr}_2\text{O}_7$ . If Mn and/or chlorides (from use of HCl, 30) are present, transfer 200 cc of filtrate to a separatory funnel, add the equivalent of 5 g of citric acid, 13(d), make slightly ammoniacal, add 5 cc of the 10% KCN soln and extract with dithizone as directed in 16, treating the extract as directed in 16(a), and finally determine the Pb electrolytically as directed in 19 and 20.

## MERCURY—TENTATIVE

(Applicable to leafy vegetables.)

## 34

## REAGENTS

(a) *Hydrogen peroxide*.—30% electrolytic soln.

(b) *Standard mercury solns*.—(1) Dissolve 500 mg of pure metal in  $\text{HNO}_3$  and dilute to 1 liter; (2) dilute 10 cc of soln (1) to 500 cc with  $\text{H}_2\text{O}$  containing a few cc of  $\text{HNO}_3$  (1 cc=0.01 mg of Hg).

(c) *Diphenylthiocarbazone* (dithizone).—Purify as directed under 13(e). Prepare as follows:

(1) *Strong soln.*—Dissolve 50 mg of dithizone in  $\text{CCl}_4$  and dilute to 100 cc.

(2) *Extraction soln.*—Measure 20 cc of soln (1) into a 200 cc volumetric flask and dilute to the mark with  $\text{CHCl}_3$ .

(3) *Titrating soln.*—Measure 2.5 cc of soln (1) into a 100 cc volumetric flask and dilute to the mark with  $\text{CCl}_4$ .

Preserve the solns in dark bottles and overlay with  $\text{H}_2\text{SO}_4(1+99)$ .

## 35

## APPARATUS

*Digestion flask and internal condenser.*—Use a 2 liter Florence flask of resistant glass, preferably Pyrex, fitted with an internal condenser, a glass cylinder about 9.5 in. long and about 1.5 in. in diameter, enlarged at the top to retain it in the neck of the flask. The bottom is cone-shaped and closed. The top may be closed with a two-holed stopper or the entire condenser may be made of glass. The condenser has an inlet tube extending nearly to the bottom, and an outlet tube. The condenser should fit closely inside the neck of the Florence flask and should extend about 1.5 in. into the body of the flask. The dimensions of the condenser will vary slightly according to the dimensions of the flask.

## 36

## DETERMINATION

Introduce into the digestion flask a suitable weighed portion of the finely chopped and well-mixed sample (100–150 g in the case of lettuce). Add 50 cc of  $\text{HNO}_3$  and 300 cc of  $\text{H}_2\text{O}$ . Place the internal condenser in the flask and start the  $\text{H}_2\text{O}$  flowing thru it.

Heat the flask over a low flame or on a hot plate until the contents boil, and reflux for 25 min. (In all refluxing the boiling must be gentle, so that the top half of the neck of the flask remains cool.) Remove the flask from the flame and cool nearly to room temp. Rinse the condenser with  $\text{H}_2\text{O}$  from a wash bottle and remove it. Filter the contents quite rapidly thru a large Büchner funnel (11–18.5 cm in diameter). Wash the flask with about 30 cc of  $\text{H}_2\text{O}$ , decanting it onto the filter when nearly all the soln has passed thru. Repeat the washing once or twice. Return the liquid to the digestion flask, washing it in with a small amount of  $\text{H}_2\text{O}$  from a wash bottle.

Digest as follows: Add 10–12 g of  $\text{KMnO}_4$ . (Add in several portions to cabbage or other foods that react vigorously or froth unduly; to lettuce extract it may be added at once.) Replace the internal condenser, and when the reaction subsides heat gently to boiling and reflux 10–15 min. Cool the flask in a bath, raise the condenser, and add 8–10 g of  $\text{KMnO}_4$  as fast as the vigor of the reaction will permit.

Replace the condenser and again heat the soln to boiling for 15 min. unless it clears in less time. Cool, add 5 or 6 g of  $\text{KMnO}_4$  and about 20 cc of  $\text{HNO}_3$ , and repeat the digestion and refluxing for about 15 min. Continue the cooling, the addition of  $\text{KMnO}_4$ , and subsequent heating until the purple color of  $\text{KMnO}_4$  persists when the liquid is heated almost to boiling. Agitate the soln during the heating to avoid bumping caused by an accumulation of black oxides of Mn. If further additions of  $\text{KMnO}_4$  are required, add more  $\text{HNO}_3$ . (All organic matter should be completely oxidized, otherwise nitrites, formed by the action of  $\text{HNO}_3$  on organic matter, will decompose the dithizone, as shown by loss of color, and thus prevent complete extraction of mercury. The oxidation is complete when the supernatant liquid appears white after the  $\text{MnO}_2$  has settled out.)

Cool, and add  $\text{H}_2\text{O}_2$  a little at a time, while shaking with a rotary motion, until the precipitated oxides of manganese dissolve completely. Be careful to insure an excess of  $\text{HNO}_3$ , otherwise a large quantity of peroxide may be used to no advantage. Replace the condenser and heat to boiling 5 min. to remove free oxygen and to dissolve refractory particles, then cool again. Add 0.5 g of crystallized hydroxylamine sulfate or chloride. (The soln should have the strength of at least 1%  $\text{HNO}_3$ .)

If antimony is present, add 15 cc of a 10% soln of tartaric acid, previously extracted several times with the dithizone soln to free it from mercury.

To concentrate the mercury and remove interfering substances, extract the liquid in portions not to exceed 425 cc as follows:

Transfer the soln to a 500 cc separatory funnel and extract with 20 cc portions of dithizone soln (2) as long as an orange yellow color persists. (The mercury-dithizone complex is a bright orange yellow color and should not be confused with the slow fading of the dithizone reagent to a greenish yellow color due to oxidation.) For each extraction shake vigorously 20–30 seconds. When a green or reddish colored extract is obtained, extract once more with 15 cc of the extraction soln. (The red is generally due to the presence of Cu and continuing the extraction further will only remove more Cu.) Draw off the extract each time into a 250 cc separatory funnel or a 250 cc beaker, depending on whether method 1 or 2 is to be used for the oxidation to follow.

If copper is present in large quantities, treat the extract as directed under *Removal of Copper*. Small quantities need not be removed.

Oxidize the combined dithizone extracts by either of the following methods:

(1) Warm the oxidizing mixture composed of 50 cc of  $\text{H}_2\text{O}$ , 10 cc of 5%  $\text{KMnO}_4$  soln and 2 cc of  $\text{H}_2\text{SO}_4$  (1+1) to 50–55° and add to the extracts in the separatory funnel. Shake gently at first, release pressure, then shake vigorously for several minutes.

(2) Evaporate the extracts to dryness on the steam bath. Add the oxidizing mixture described in (1) and allow to remain on the steam bath for 15–20 min.

Add sufficient 10%  $\text{NaNO}_2$  soln dropwise to clear the soln obtained under (1) or (2). Shake or stir after the addition of a few drops. Discard the  $\text{CHCl}_3$  portion in (1). To the clear soln add 0.75 g of hydroxylamine sulfate or chloride and warm to 60°. Add  $\text{KMnO}_4$  to the standard and clear it in the same manner later, before making the titration.

Add  $\text{HCl}$  if Ag is present or suspected. If only a minute quantity of Hg is expected, use the entire soln for the determination and titrate as directed later. When larger quantities of Hg (0.1–3 mg) are expected, transfer the liquid to a suitable volumetric flask and determine the Hg in an aliquot as directed below.

*Small quantities.*—Before beginning the titration, fill two burets with the dithizone titrating soln and place 10 cc of the standard Hg soln in another separatory funnel. Dilute to about 50 cc with  $\text{H}_2\text{O}$  and add 2 cc of  $\text{H}_2\text{SO}_4$  (1+1) and 10 cc of 5%  $\text{KMnO}_4$  soln, and clear as previously directed. Add to the sample from buret No. 1, 5 cc of dithizone soln, close the funnel, shake a few times, then invert and open the stopcock to relieve the pressure. Close the funnel and shake vigorously for 10 seconds. Allow the funnel to stand until the liquids separate. (By giving the funnel a rotary whirl, drops collecting on the sides will usually go to the bottom.)

To the standard soln in the other funnel, add 1 cc of dithizone soln from buret No. 2, shake, and allow to stand until the layers separate. Compare the colors of the  $\text{CCl}_4$  layers in the two funnels. (The Hg compound should be bright orange yellow, easily recognized after a little experience.) Continue adding dithizone soln



in 4 cc portions (read the buret each time), shaking as above after each addition, and after each second addition drawing off the  $\text{CCl}_4$  layer into another separatory funnel, until on separation the yellow color due to Hg no longer appears but is supplanted by another color, generally the green of the reagent, indicating the end point is past. Then add sufficient standard Hg soln (usually 1 cc containing 0.01 mg is sufficient) to the titrated sample to supply an excess of Hg, shake, and draw off the extract. Titrate the excess of Hg with the dithizone soln, adding a few tenths cc of the soln at a time. Subtract the Hg added. Titrate the standard in the same manner and calculate the Hg in the sample.

*Large quantities.*—Titrate large quantities similarly, using a new aliquot for the exact titration.

*Removal of copper.*—Ordinarily, Cu does not interfere. If necessary remove as follows: Shake the combined dithizone extracts with 60 cc of an  $\text{H}_2\text{O}$  soln containing a few drops of  $\text{H}_2\text{SO}_4$  (1+1), a few crystals of KI, and a few drops of a 5% soln of Na arsenite to prevent the liberation of free I. Shake vigorously about 20 seconds and carefully draw off the dithizone layer. Wash the aqueous soln with a little  $\text{CHCl}_3$ . (This treatment leaves the Cu in the extract and transfers the Hg to the aqueous phase.)

Use either of the two following procedures for the extraction: (1) Make the soln ammoniacal and extract with dithizone until the orange yellow compound no longer forms; (2) extract the mercury from the acid soln containing iodides by adding 2 cc of a 1% soln of Na diethyldithiocarbamate and using several 10 cc portions of  $\text{CHCl}_3$ . Then oxidize the extracts from (1) or (2) and titrate as previously directed.

## TIN

37

## PREPARATION OF SAMPLE

Digest a 50–100 g sample as directed under 3.

38

*Gravimetric Method<sup>8</sup>—Tentative*

Add 200 cc of  $\text{H}_2\text{O}$  to the digested sample and transfer to a 600 cc beaker. Rinse the Kjeldahl flask with 3 portions of boiling  $\text{H}_2\text{O}$ , making a total volume of approximately 400 cc. Cool, and add  $\text{NH}_4\text{OH}$  until just alkaline, then 5 cc of  $\text{HCl}$  or 5 cc of  $\text{H}_2\text{SO}_4$  (1+3) for each 100 cc of soln. Place the beaker, covered, on a hot plate; heat to about  $95^\circ$  and pass in a slow stream of  $\text{H}_2\text{S}$  for an hour. Digest at  $95^\circ$  for an hour and allow to stand 30 min. longer.

Filter, and wash the precipitate of  $\text{SnS}$  alternately with 3 portions each of wash soln (100 cc of saturated  $\text{NH}_4$  acetate soln, 50 cc of glacial acetic acid, and 850 cc of  $\text{H}_2\text{O}$ ) and hot  $\text{H}_2\text{O}$ . Transfer the filter and precipitate to a 50 cc beaker, add 10–20 cc of  $\text{NH}_4$  polysulfide, heat to boiling, and filter. Repeat the digestion with  $\text{NH}_4$  polysulfide and the filtration twice, and then wash the filter with hot  $\text{H}_2\text{O}$ . Acidify the combined filtrate and washings with acetic acid (1+9), digest on a hot plate for an hour, allow to stand overnight, and filter thru a double 11 cm filter. Wash alternately with 2 portions each of the wash soln and hot  $\text{H}_2\text{O}$  and dry thoroly in a weighed porcelain crucible. Ignite over a Bunsen flame, very gently at first to burn off filter paper and to convert the sulfide to oxide; then partly cover the crucible and heat strongly over a large Bunsen or Meker burner. ( $\text{SnS}$  must be roasted gently to the oxide, which may be heated to a high temp. without loss by volatilization.) Weigh as  $\text{SnO}_2$  and calculate to metallic tin, using the factor 0.7877.

Volumetric Method<sup>1</sup>—Tentative

39

## REAGENTS

(a) *Air-free wash soln.*—Dissolve 20 g of  $\text{NaHCO}_3$  in 2 liters of boiled  $\text{H}_2\text{O}$  and add 40 cc of  $\text{HCl}$ . This soln should be freshly prepared.

(b) *Iodine.*—0.01 *N*. Standardize the soln frequently against (c), adding an asbestos mat and proceeding as described under 40, omitting the precipitation with  $\text{H}_2\text{S}$  and the boiling with  $\text{HCl}$  and  $\text{KClO}_4$ . The quantity of  $\text{Sn}$  in the soln used for the standardization should equal approximately that contained in the sample under examination.

(c) *Standard tin soln.*—Dissolve 1 g of  $\text{Sn}$  in about 500 cc of  $\text{HCl}$  and dilute to 1 liter with  $\text{H}_2\text{O}$ . 1 cc contains 1 mg of  $\text{Sn}$ .

(d) *Sheet aluminum.*—About 30 gage, free from  $\text{Sn}$ .

40

## DETERMINATION

Proceed as directed under 38 to "Digest at 95° for an hour and allow to stand 30 min. longer."

Filter thru asbestos in a Gooch crucible having a detachable bottom, using suction. Wash the precipitate of  $\text{SnS}$  a few times and then transfer the detachable bottom and asbestos pad to a 300 cc Erlenmeyer flask. Remove all traces of the precipitate from the inside of the crucible by means of a jet of hot  $\text{H}_2\text{O}$  and a rubber-tipped rod, using a minimum quantity of  $\text{H}_2\text{O}$  for washing.

Add to the flask 100 cc of  $\text{HCl}$  and 0.5 g of  $\text{KClO}_3$ . Boil for approximately 15 min., making about 4 more additions of smaller quantities of the  $\text{KClO}_3$  as  $\text{Cl}$  is boiled out of the soln. Wash the particles of  $\text{KClO}_3$  down from the neck of the flask with  $\text{H}_2\text{O}$  and finally boil to remove  $\text{Cl}$ . Then add about 1 g of the sheet  $\text{Al}$  to dispel the last traces of  $\text{Cl}$ .

Fit a 2-holed rubber stopper to the flask. Thru one of the holes pass a bulbod glass tube that reaches nearly to the surface of the liquid. Attach this tube to a large  $\text{CO}_2$  generator thru a scrubber containing  $\text{H}_2\text{O}$ . The  $\text{CO}_2$  passes out of the flask thru a short, bulbod tube inserted in the second hole of the rubber stopper and terminating slightly below it. Connect this second glass tube by means of a rubber tube with another glass tube, approximately 10 in. long, which is immersed in a cylinder of  $\text{H}_2\text{O}$  to a depth of approximately 8 in. This connection will act as a seal to restrain any strong flow of gas when not desired and to permit a pressure in the flask.

Raise the delivery tube nearly out of the  $\text{H}_2\text{O}$  seal, thus allowing a rapid flow of  $\text{CO}_2$  for a few minutes to dispel the air from the system. Then lower the delivery tube into the  $\text{H}_2\text{O}$  seal, slightly raise the stopper, and quickly drop into the flask 1-2 g of  $\text{Al}$  foil, folded into a narrow bent strip to prevent breaking the flask. When the  $\text{Al}$  has completely dissolved, raise the tube in the  $\text{H}_2\text{O}$  seal, allowing the  $\text{CO}_2$  to pass thru rapidly; place the flask upon a hot plate and boil for a few min. Remove the flask from the heat and cool with tap or ice  $\text{H}_2\text{O}$ , continuing the flow of  $\text{CO}_2$ . Lower the delivery tube into the cylinder, disconnect the flask, and, with a glass plug, close the rubber tube thru which the  $\text{CO}_2$  enters the flask. Wash the glass tubes, rubber stopper, and sides of the flask with the air-free wash soln; add starch indicator, VI, 3(e), and titrate immediately with the 0.01 *N*  $\text{I}$  soln.

If desired, the titration may be made by slightly raising the rubber stopper after cooling and adding an excess of the 0.01 *N*  $\text{I}$  soln. Then disconnect the flask; wash

the tubes, rubber stopper, and sides of flask with the air-free wash soln; and titrate the excess of I with 0.01 *N*  $\text{Na}_2\text{S}_2\text{O}_3$ .

#### COPPER<sup>3</sup>

41

##### PREPARATION OF SAMPLE

Digest a 50–100 g sample as directed under 3, or ash it as described under 14. The sample should contain a minimum of 1 mg of copper and 2 mg of Zn, if Zn also is to be determined.

##### *Volumetric Method—Tentative*

42

##### REAGENTS

(a) *Standard copper soln.*—Dissolve 63.6 mg of pure metallic Cu in  $\text{HNO}_3$  and evaporate to dryness on a steam bath. Add sufficient  $\text{H}_2\text{O}$  and a few drops of acetic acid to dissolve the  $\text{Cu}(\text{NO}_3)_2$  and again evaporate to dryness on a steam bath. Redissolve the  $\text{Cu}(\text{NO}_3)_2$  as above and make up to 1 liter.

(b) *Sodium thiosulfate soln.*—Dissolve 24.82 g of  $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$  in 1 liter of  $\text{CO}_2$ -free  $\text{H}_2\text{O}$  to make an approximate 0.1 *N* soln. Allow to stand, preferably for about 2 weeks. Prepare 0.005 or 0.01 *N* solns by dilution of this reagent with  $\text{CO}_2$ -free  $\text{H}_2\text{O}$  in the ratio of 1:20 or 1:10. Standardize daily against the standard Cu soln in the following manner: Place 20 cc of the standard Cu soln in a 100 cc Erlenmeyer flask, add an excess of  $\text{NH}_4\text{OH}$ , and continue as directed under 43, beginning with the words, "and boil gently to drive off excess ammonia." 1 cc of 0.01 *N*  $\text{Na}_2\text{S}_2\text{O}_3 = 0.6357$  mg of Cu.

43

##### DETERMINATION

Dissolve the ashed sample in  $\text{HCl}$  and neutralize this soln, or neutralize the soln obtained by the wet digestion, with  $\text{NH}_4\text{OH}$ . Add 5 cc of  $\text{H}_2\text{SO}_4$ , dilute the soln to 200 cc, and boil for 1 min. Then add cautiously 10 cc of a hot saturated soln of  $\text{Na}_2\text{S}_2\text{O}_3$  and continue the boiling for 5 min. (With larger quantities of Cu the precipitate coagulates, and the liquid becomes practically clear. A few cc of 1%  $(\text{NH}_4)_2\text{SO}_4$  soln may be added to hasten the coagulation.) Filter the precipitate and wash 6 times with hot  $\text{H}_2\text{O}$ . Reserve the filtrate for the determination of Zn, if necessary. Fold the precipitate within the filter paper, place in a small crucible, and ignite in an electric muffle at about  $500^\circ$  (a barely visible red heat). Treat the residue with 1 cc of  $\text{HNO}_3$  (2+5) and dry on the steam bath. Add 5 cc of  $\text{H}_2\text{O}$  and again evaporate to dryness on the steam bath. Add 20 cc of  $\text{H}_2\text{O}$ , an excess of  $\text{NH}_4\text{OH}$ , and heat on the steam bath until Cu salts are dissolved. Transfer to a 100 cc Erlenmeyer flask and boil gently to drive off excess ammonia. Make acid to litmus paper with acetic acid (1+1), add 1 cc in excess, boil the soln 1 min. and cool to room temp. Add 2 g of KI dissolved in enough  $\text{H}_2\text{O}$  to make the final soln 50 cc, and titrate the free I immediately with 0.01 *N* or 0.005 *N*  $\text{Na}_2\text{S}_2\text{O}_3$  (according to the amount of Cu present, as shown by the degree of blue color in the ammoniacal soln) until the end point is nearly reached. Then add 2 cc of starch soln, VI, 3(e), and continue the titration dropwise to the same shade of whiteness obtained in titrating the standard.

#### ZINC

##### *Gravimetric Method—Tentative*

44

##### REAGENTS

(a) *Sodium or ammonium acetate soln.*—Dissolve 50 g of the salt in  $\text{H}_2\text{O}$  and make up to 100 cc.

(b) *Ferric chloride soln.*—Dissolve 10 g of  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  in 100 cc of  $\text{H}_2\text{O}$ .

## 45

## DETERMINATION

Boil the filtrate containing the Zn obtained after filtering off the copper sulfide (43) to expel  $\text{H}_2\text{S}$  and reduce the volume to 250–300 cc, add a drop of methyl orange indicator, 5 g of  $\text{NH}_4\text{Cl}$ , and make alkaline with  $\text{NH}_4\text{OH}$ . Add  $\text{HCl}$  (1+9) dropwise to faintly acid reaction, then add 10–15 cc of the Na or  $\text{NH}_4$  acetate soln and pass in  $\text{H}_2\text{S}$  until precipitation is complete. Allow the precipitate to settle, filter (a clear filtrate is necessary), and wash the precipitate twice with  $\text{H}_2\text{S}$  water. Dissolve the precipitate on the filter with a little  $\text{HCl}$  (1+3), wash the filter with  $\text{H}_2\text{O}$ , boil the combined filtrate and washings to expel  $\text{H}_2\text{S}$ , cool, and add a distinct excess of Br water. Add 5 g of  $\text{NH}_4\text{Cl}$  and then  $\text{NH}_4\text{OH}$  until the color of free Br disappears. Add  $\text{HCl}$  (1+3) dropwise until the Br color just reappears; then add 10–15 cc of the Na or  $\text{NH}_4$  acetate soln and 0.5 cc of the  $\text{FeCl}_3$  soln, or enough to precipitate all the phosphates. Boil until all the iron is precipitated. Filter while hot and wash the precipitate with  $\text{H}_2\text{O}$  containing a little Na acetate. Pass  $\text{H}_2\text{S}$  into the combined filtrate and washings until all the  $\text{ZnS}$ , which should be pure white, is precipitated. Filter thru a weighed Gooch crucible, previously heated to constant weight, and wash with  $\text{H}_2\text{S}$  water containing a little  $\text{NH}_4\text{NO}_3$ . Dry the crucible and its contents in an oven, ignite at a bright red heat, cool, and weigh as  $\text{ZnO}$ . Calculate the weight of metallic Zn, using the factor 0.8034.

## 46

## MANGANESE—TENTATIVE

Proceed according to either of the official methods for the determination of Mn given under XII.

## SELECTED REFERENCES

- <sup>1</sup> U. S. Dept. Agr. Bur. Chem. Circ., 102 (1912); J. Assoc. Official Agr. Chem., 7, 48 (1923); 16, 398 (1933); 18, 189, 506 (1935); 19, 95 (1936).
- <sup>2</sup> J. Assoc. Official Agr. Chem., 16, 75 (1933); 17, 70, 202 (1934); 19, 95 (1936).
- <sup>3</sup> Ibid., 17, 108 (1934); 18, 315 (1935); 19, 130 (1936).
- <sup>4</sup> Ind. Eng. Chem. Anal. Ed., 7, 285 (1935).
- <sup>5</sup> J. Assoc. Official Agr. Chem., 18, 638 (1935).
- <sup>6</sup> Ibid., 1, 257 (1915).
- <sup>7</sup> Original Communications, VIII Intern. Cong. Appl. Chem., 18, 35 (1912).
- <sup>8</sup> J. Assoc. Official Agr. Chem., 13, 426 (1930).

### XXX. NUTS AND NUT PRODUCTS<sup>1</sup>

1

#### PREPARATION OF SAMPLE—TENTATIVE

Without delay, transfer all samples received in packages to glass-stoppered containers or Mason jars and keep in a cool, dark place. Prepare the various samples for analysis as follows:

(a) *Fresh shelled nuts*.—Cut the nuts into small pieces and weigh the sample desired for analysis. Transfer the weighed sample to a mortar and grind to a fine state of division with a pestle. In transferring the ground material from the mortar to the required flask, use a portion of the solvent to clean out the mortar.

(b) *Shredded or prepared coconut*.—Transfer the weighed sample to a mortar and proceed as directed under (a).

(c) *Almond paste, kernel paste, peanut butter, etc.*—Transfer the sample to a Mason jar or beaker about three times the size of the sample and mix carefully with a stiff-bladed spatula or knife. *Almond paste and similar products containing added H<sub>2</sub>O must be freed of moisture before analysis.*

#### MOISTURE

2

##### Method I.—Tentative

Proceed as directed in XXVII, 3.

3

##### Method II.—Tentative

Weigh 2 g of the product into a flat-bottomed dish. If necessary to secure a thin layer of the material, add a few cc of H<sub>2</sub>O, and mix thoroly. Dry at 70° under a pressure not to exceed 100 mm of mercury until consecutive weighings made at intervals of 2 hours do not vary more than 3 mg.

4

#### ASH—TENTATIVE

Proceed as directed under XXVII, 8.

5

#### CRUDE FIBER—TENTATIVE

Determine crude fiber on the fat-free material as directed under XXVII, 27.

#### FAT, CONSTANTS OF FAT, AND PROTEIN

6

##### Method I.—Tentative

Weigh into a 200 cc volumetric flask 2–3 g of material. Add 100 cc of chloroform from a pipet, washing down the sample with a stream of chloroform. Stopper the flask and shake frequently during 30 min. Filter the soln thru an 11 cm fluted filter paper, and as soon as 25 cc of the soln has been filtered, pipet out two 10 cc portions, using the same pipet. Transfer one aliquot to a weighed crystallizing dish, 50 × 35 mm, and evaporate the solvent on a steam bath. Dry the dish and contents at 100° for 30 min., cool, and weigh. Use the weight obtained for calculating the percentage of fat and the iodine number of the fat. Determine the refractive index of the dried residue. Transfer the other 10 cc portion to a glass-stoppered flask or bottle, add 30 cc of Hanus soln, XXXI, 18(a), and proceed as directed under XXXI, 18. Complete the filtration of the chloroform extract. Transfer the extracted residue to a Kjeldahl flask, wash the volumetric flask thoroly with boiling H<sub>2</sub>O, and transfer the washings to the digestion flask. Determine N as directed under II, 25.  $N \times 6.25 = \text{protein}$ .

7

*Method II.—Tentative*

Proceed as directed under XIX, 12, using a 300 cc Erlenmeyer receiving flask in place of the 150 cc flask. (A fritted Jena glass Büchner filter is more convenient than the Knorr extraction tube.) To facilitate filtration, mix an equal volume of filter cel with peanut butter.

Determine constants of fat and protein as directed under 6.

**SUGAR AND SALT**

8

*Method I.—Tentative*

Extract approximately 10 g of the sample in an 8 oz nursing bottle with two 100 cc portions of petroleum ether, in each case shaking for 5 min., centrifuging, and pouring off the supernatant liquid. Warm the bottle to drive off the remaining solvent and transfer the dry residue to a 100-150 cc separatory funnel. Complete the transfer with a mixture of 3 volumes of carbon tetrachloride and one volume of chloroform. Add more of the liquid and shake the mixture vigorously. Wash down the sides of the funnel, using a total quantity of 60-80 cc of the liquid. Stopper the funnel and allow to stand overnight. Remove the sugar and salt that have settled out by opening the stopcock quickly, and if necessary pull out the stopcock. Evaporate off the liquid on the steam bath or other warm place and dissolve the residue in hot H<sub>2</sub>O. Transfer the soln to a 100 cc volumetric flask with hot H<sub>2</sub>O, cool, make up to the mark, and mix. Filter thru a small, dry filter paper. Determine chlorides in a 20 cc aliquot by titration with AgNO<sub>3</sub>, using dichromate indicator. Determine reducing sugars before and after inversion as directed under XXXIV, 37.

9

*Method II.—Tentative*

Weigh 10 g of the material on a filter (with peanut butter thoroly mix 5 g of filter cel with the weighed charge) and extract with suction 10 successive times at 3 min. intervals with 50 cc of petroleum ether (b.p. below 60°). (A fritted Jena glass Büchner filter is most convenient.) At the beginning of each extraction stir the soln well with a glass rod flattened at the end. After defatting, macerate well in a porcelain mortar and transfer the material with hot H<sub>2</sub>O to a 250 cc Pyrex or similar volumetric flask. If frothing occurs, add a few drops of caprylic alcohol, breaking up the foam with a glass rod. Pass hot H<sub>2</sub>O thru the filter and add to the H<sub>2</sub>O in the flask until the total volume is about 200 cc. Digest in a vessel of boiling H<sub>2</sub>O for 15 min., cool under the tap, and add about 5 cc of a saturated neutral Pb acetate soln. Make to the mark at room temp., shake well, transfer to a centrifuge bottle, and whirl at 2000 r.p.m. for 15 min. Filter on an 18½ cm folded filter, rejecting the first 25 cc of the filtrate. De-lead with potassium oxalate and again filter, rejecting the first 25 cc of the filtrate. Determine the reducing sugars before and after inversion and multiply by 0.95 to obtain the sucrose. Multiply the results obtained by 0.97 to correct for the volume occupied by the almond paste and coconut. In the case of peanut butter, multiply by the factor 0.95 to correct for the volume occupied by the filter cel and peanut butter. Determine chlorides as directed under 8.

10

**DEXTROSE OR D-GLUCOSE—TENTATIVE**

Proceed as directed under 8 or 9 and XXXIV, 37.

**PEANUT BUTTER**

11

**PRELIMINARY PROCEDURE—TENTATIVE**

Make a microscopical examination to detect the addition of starch or any off-grade material not identifiable chemically.

## 12

## STARCH—TENTATIVE

Weigh 4–5 g of the sample by difference into an 8 oz nursing bottle and extract twice with 50 cc portions of petroleum ether, in each case shaking for 5 min. Wash down the sides of the bottle with petroleum ether, centrifuge, and pour off the solvent, disregarding opalescence. Warm the bottle to drive off the remaining solvent, and transfer the residue to a mortar and grind. Return the fine powder to the bottle with the aid of 100 cc of 10% NaCl soln. Shake the bottle for 15 min., wash down the sides with salt soln, centrifuge well, and pour off the supernatant liquid, disregarding opalescence. Repeat this procedure twice. Extract once in the same manner with 70% alcohol and then once with  $H_2O$ , shaking for 1–2 min. in each case. Drain the bottle for several minutes, then chill and add from a pipet 100 cc of HCl soln (20.5–21.0 g of HCl per 100 cc) at a temp. not higher than 15°. Shake vigorously for 3 min., centrifuge well, and pour off the soln thru a pledget of cotton in the stem of a funnel. Cool the soln to the temp. at which the HCl was added, and pipet off 50 cc into a nursing bottle containing 115 cc of 95% alcohol. Shake with a whirling motion for 1 min., let stand for 2 min., centrifuge for 2 min., pour off thru a weighed Gooch crucible containing a thin pad of asbestos, and add 50 cc of 70% alcohol to the precipitate. Stopper the bottle, shake vigorously, wash down the sides with the alcohol, centrifuge lightly, and pour off thru the crucible. Repeat once with 70% alcohol and once with 95% alcohol. Dry the crucible and contents for 1.5 hours at 130° in air, or for 5 hours at 98–100° in vacuo. Cover the crucible, place in a desiccator containing an efficient desiccant, and weigh the crucible as soon as it has attained room temp.

## ALMOND PASTE, KERNEL PASTE, ETC.

## 13

## SEPARATION AND PREPARATION OF THE OIL—TENTATIVE

Dry the paste in an oven and extract repeatedly with petroleum ether by rubbing in a mortar and pouring off the solvent thru a filter. Evaporate the ether on a steam bath and test the extracted oil.

## 14

*Bieber's Test<sup>1</sup>*

Agitate 5 volumes of oil with 1 volume of a mixture of equal parts, by weight, of  $H_2SO_4$ , fuming  $HNO_3$ , and  $H_2O$ . Pure almond oil does not change color; after standing for some time apricot kernel oil gives a pink peach-blossom color, and peach kernel oil, a faint pink coloration. It is advisable to prepare the reagent fresh for each set of tests. It is doubtful whether less than 25% of apricot kernel oil can be detected.

## 15

*Nitric Acid Test<sup>2</sup>*

On being shaken with nitric acid, almond oil remains colorless or becomes slightly yellow; apricot kernel oil assumes a color ranging from orange-yellow to red; and peach kernel oil becomes a yellowish brown.

## 16

*Kreis Test<sup>3</sup>*

Mix 1 volume of the oil in a test tube with 1 volume of a 0.10% soln of phloroglucinol in ether, and pour 1 volume of  $HNO_3$  down the side of the tube. Keep the tube cold. A red ring forms at the junction of the two liquids when apricot kernel, sesame, or cottonseed oil is present. Almond oil gives no red color—or, at most, only a light pink.

The presence or absence of other oils (such as cottonseed, sesame, peanut, or olive) may be detected by the variation in constants and by characteristic tests. It is seldom that these oils are found unless added starch is present.

## 17

## MICROSCOPIC EXAMINATION

In connection with the microscopic examination of almond paste and other products containing ground almonds, attention is called to the following publications, which give detailed descriptions and illustrations of the tissue elements:

Young, W. J.—A Study of Nuts with Special Reference to Microscopic Identification. U. S. Dept. Agr. Bur. Chem. Bull. 160 (1912).

Hamig, E.—*Z. Nahr. Genussm.*, 21, 577 (1911).

Pease, V. A.—Notes on the Histology of the Almond, *J. Agr. Research*, 41, 789-800 (1930).

Winton, Andrew L. and Kate B.—The Structure and Composition of Foods, Vol. 1, p. 476 (1932).

## SHREDDED COCONUT

## 18

## GLYCEROL—TENTATIVE

Extract with suction 4 times, 4 g of the shredded coconut (dried in vacuo at 70° for 5 or 6 hours) on a filter (a fritted Jena glass Büchner filter is most convenient), using for each extraction 50 cc of petroleum ether (b.p. below 65°), and allowing 3 min. intervals between extractions. Use a flattened glass rod for stirring. After removing the fat, extract the residue on the filter with four 50 cc portions of absolute alcohol, allowing 3 min. intervals with stirring, as before. Make the absolute alcohol extract to 250 cc with absolute alcohol at room temp. Pipet 100 cc into a 500 cc Erlenmeyer flask, and add 5 cc of H<sub>2</sub>O and a paste made by adding hot H<sub>2</sub>O to 2 or 3 g of Ba(OH)<sub>2</sub> in a small mortar. Heat the mixture on a steam bath to boiling and boil for about 1 min.; transfer to a 250 cc centrifuge bottle, and centrifuge at 2000 r.p.m. for about 5 min. Transfer the clear liquid to a large porcelain dish and wash the residue in the centrifuge bottle with 50-75 cc of absolute alcohol, stirring with a glass rod and centrifuging as before. Evaporate on a steam bath at a temp. below 70° to a few drops, or almost dryness. Transfer to a 50 cc glass-stoppered cylinder with 10 cc of absolute alcohol and wash the dish with two 5 cc portions of absolute alcohol. Further wash the dish with three 10 cc portions of anhydrous ether, shaking the glass-stoppered cylinder thoroly after each addition of the anhydrous ether. Transfer to a sediment tube and centrifuge for 10 min. at a speed of 3200 r.p.m. Transfer the clear soln in the sediment tube to an evaporating dish, preferably platinum, and wash the sediment tube with 25 cc of a mixture of absolute alcohol and anhydrous ether (2:3), stirring with a glass stirring rod and centrifuging as before. Evaporate on the steam bath at a temp. of 85-90° to about 5 cc, add 20 cc of H<sub>2</sub>O, and evaporate to about 5 cc; repeat this operation twice. Transfer the residue with hot H<sub>2</sub>O to a 50 cc volumetric flask and proceed as directed under XXXIII, 72.

## SELECTED REFERENCES

<sup>1</sup> J. Assoc. Official Agr. Chem., 18, 419 (1935).

<sup>2</sup> Z. Anal. Chem., 17, 264 (1878); Pharm. Centralb., 18, 315.

<sup>3</sup> Schweizer Lebensmittelbuch, 3rd ed., p. 43 (1917).

<sup>4</sup> Chem. Ztg., 26, 897 (1902).



## XXXI. OILS, FATS, AND WAXES

1

### PREPARATION OF SAMPLE—OFFICIAL

Melt solid fats and filter by means of a hot water funnel or similar apparatus. Make the different determinations on samples of this melted, homogeneous mass. Filter oils that are not clear. Keep oils and fats in a cool place and protected from light and air, otherwise they will soon become rancid. Weigh out at one time as many portions as are needed for the various determinations, using a small beaker or weighing buret.

### MOISTURE AND VOLATILE MATTER<sup>1</sup>

2

#### *Vacuum Oven Method—Official*

Soften the sample if necessary by means of gentle heat, taking care not to melt it. When sufficiently softened, mix thoroly with a mechanical egg beater or other equally effective mechanical mixer.

Weigh 5 g ( $\pm 0.2$  g) of the prepared sample into a shallow glass moisture dish approximately 6–7 cm in diameter and 4 cm deep. Dry to constant weight in a vacuum oven (F.A.C. standard or equivalent) at a uniform temp. not less than  $20^{\circ}$  nor more than  $25^{\circ}$  above the boiling point of  $H_2O$  at the working pressure, which should not exceed 100 mm of mercury. Constant weight is attained when successive dryings for 1 hour periods show an additional loss of not more than 0.05%. Cool the sample in an efficient desiccator (30 min.) and reweigh. Report the percentage loss in weight as moisture and volatile matter.

### SPECIFIC GRAVITY (APPARENT)

#### *At $25/25^{\circ}$ —Official*

3

### STANDARDIZATION OF PYCNOMETER

Carefully clean the pycnometer by filling it with a saturated soln of  $CrO_3$  in  $H_2SO_4$  and allowing to stand for several hours. Empty the pycnometer and rinse thoroly with  $H_2O$ ; then fill it with recently boiled  $H_2O$  previously cooled to about  $20^{\circ}$  and place in a constant temp. bath at  $25^{\circ}$ . At the end of 30 min. adjust the level of the  $H_2O$  to the proper point on the pycnometer and put the perforated cap or stopper in place; remove from the bath, wipe dry with a clean cloth or towel, allow to stand for 30 min., and weigh. Empty the pycnometer, rinse several times with alcohol and then with ether, allow it to become perfectly dry, remove ether vapor, and weigh. Ascertain the weight of contained  $H_2O$  at  $25^{\circ}$  by subtracting the weight of the pycnometer from its weight when full.

4

### DETERMINATION

Fill the clean, dry pycnometer with the oil previously cooled to about  $20^{\circ}$ , place in a constant temp. bath at  $25^{\circ}$  for 30 min., adjust the level of the oil to the proper point on the pycnometer, and put the cap or stopper in place; remove from the bath, wipe dry, and weigh as directed under 3. Subtract the weight of the empty pycnometer from its weight when filled with oil and divide the difference by the weight of  $H_2O$  at  $25^{\circ}$ , as determined under 3. The quotient is the sp. gr. at  $25/25^{\circ}$  (apparent).

## 5 TEMPERATURE CORRECTION FOR SPECIFIC GRAVITY OF OILS—OFFICIAL

If the sp. gr. of the oil is determined at other than standard temp., the approximate sp. gr. at 25° may be calculated by means of the following formula:

$$G = G' + 0.0007 (T - 25^\circ), \text{ in which}$$

$$G = \text{sp. gr. at } 25^\circ;$$

$$G' = \text{sp. gr. at } \frac{T}{25^\circ};$$

$$T = \text{temp. at which the sp. gr. was determined; and}$$

$$0.0007 = \text{mean correction}^2 \text{ for } 1^\circ.$$

*At the Temperature of Boiling Water—Official*

## 6 STANDARDIZATION OF FLASKS

(a) Weigh a 25–30 cc sp. gr. flask and fill with freshly boiled hot H<sub>2</sub>O. Place in a briskly boiling water bath for 30 min., replacing any evaporation from the flask by the addition of boiling H<sub>2</sub>O. Then insert the stopper, previously heated to 100°, remove the flask, cool, and weigh.

(b) The following formula may be used for calculating the weight of H<sub>2</sub>O ( $W^T$ ) which a given flask will hold at  $T^\circ$  (weighed in air with brass weights at the temp. of the room) from the weight of H<sub>2</sub>O ( $W^t$ ) (weighed in air with brass weights at the temp. of the room) contained therein at  $t^\circ$ :

$$W^T = W^t \frac{d^T}{d^t} [1 + 0.000026 (T - t)], \text{ in which}$$

$$d^T = \text{the density of H}_2\text{O at } T^\circ; \text{ and}$$

$$d^t = \text{the density of H}_2\text{O at } t^\circ.$$

## 7 DETERMINATION

Fill the dry flask with the dry, hot, freshly filtered fat, which should be entirely free from air bubbles, and keep in a water bath for 30 min. at the temp. of boiling H<sub>2</sub>O. Insert the stopper, previously heated to 100°, cool, and weigh. Divide the weight of contained fat by the weight of contained H<sub>2</sub>O previously found to obtain the sp. gr.

The weight of H<sub>2</sub>O at boiling temp. must be determined under the barometric conditions prevailing at the time the determination is made.

## INDEX OF REFRACTION

## 8 GENERAL DIRECTIONS—OFFICIAL

Place the instrument in such a position that diffused daylight or some form of artificial light can readily be obtained for illumination. Circulate thru the prisms a stream of H<sub>2</sub>O of constant temp. Determine the index of refraction with any standard instrument, reading oils at 20° and fats at 40°. The readings of the Zeiss butyrorefractometer on fats may be reduced to standard temp. by the following formula:<sup>3</sup>

$$R = R' + 0.55 (T' - T), \text{ in which}$$

$$R = \text{the reading reduced to temp. } T;$$

$$R' = \text{the reading at } T';$$

$$T' = \text{the temp. at which reading } R' \text{ is made;}$$

$$T = \text{the standard temp.; and}$$

$$0.55 = \text{correction in scale divisions for } 1^\circ.$$

With oils the factor 0.58 is substituted in the formula for 0.55, because they have a higher index of refraction. The readings of instruments that give the index of refraction directly can be reduced to standard temp. by substituting the factor 0.00038 for 0.55 in the formula. As the temp. rises the refractive index falls. The instrument used may be standardized with  $H_2O$  at  $20^\circ$ , the theoretical refractive index of  $H_2O$  at that temp. being 1.3330. Any correction found should be made on all readings. The index of refraction varies with the sp. gr. and in the same direction. If the results appear abnormal, compare the specific refractive power<sup>4</sup> with the normal.

Calculate the specific refractive power from the formula  $\frac{N-1}{D}$ , in which  $N$  equals

the refractive index and  $D$  the sp. gr. According to Proctor,<sup>1</sup> the Lorenz formula,

$$\frac{N^2-1}{(N^2+2)D},$$

gives much more satisfactory results than  $\frac{N-1}{D}$ .

## 9

*I. By Means of the Abbé Refractometer—Official*

To charge the instrument, open the double prism by means of the screw head and place a few drops of the sample on the prism or, if preferred, open the prisms slightly by turning the screw head and pour a few drops of the sample into the funnel-shaped aperture between the prisms. Then close the prisms firmly by tightening the screw head. Allow the instrument to stand for a few min. before the reading is made, so that the temp. of the sample and the instrument will be the same.

The method of measurement is based upon the observation of the position of the *border line of total reflection* in relation to the faces of a prism of flint glass. Bring this border line into the field of vision of the telescope by rotating the double prism by means of the alidade in the following manner: Hold the sector firmly and move the alidade backward or forward until the field of vision is divided into a light and a dark portion. The line dividing these portions is the "border line," and, as a rule, will not be a sharp line but a band of color. The colors are eliminated by rotating the screw head of the compensator until a sharp, colorless line is obtained. The border line should now be adjusted so that it falls on the point of intersection of the cross hairs. Read the refractive index of the substance directly on the scale of the sector. Check the correctness of the instrument as directed under 8, or by means of the quartz plate that accompanies it, using monobromonaphthalene, and make the necessary correction in the reading.

## 10

*II. By Means of the Zeiss Butyro-Refractometer—Official*

Place 2 or 3 drops of the filtered fat on the surface of the lower prism. Close the prisms and adjust the mirror until it gives the sharpest reading. If the reading is indistinct after running  $H_2O$  of a constant temp. thru the instrument for some time, the fat is unevenly distributed on the surfaces of the prism. As the index of refraction is greatly affected by temp., use care to keep the latter constant. Carefully adjust the instrument by means of the standard fluid that is supplied with it. Convert the degrees of the instrument into refractive indices from the table under 11.

11

*Butyro-refractometer readings and indices of refraction*

READING	INDEX OF REFRACTION	READING	INDEX OF REFRACTION	READING	INDEX OF REFRACTION	READING	INDEX OF REFRACTION
40.0	1.4524	50.0	1.4593	60.0	1.4659	70.0	1.4723
40.5	1.4527	50.5	1.4596	60.5	1.4662	70.5	1.4726
41.0	1.4531	51.0	1.4600	61.0	1.4665	71.0	1.4729
41.5	1.4534	51.5	1.4603	61.5	1.4668	71.5	1.4732
42.0	1.4538	52.0	1.4607	62.0	1.4672	72.0	1.4735
42.5	1.4541	52.5	1.4610	62.5	1.4675	72.5	1.4738
43.0	1.4545	53.0	1.4613	63.0	1.4678	73.0	1.4741
43.5	1.4548	53.5	1.4616	63.5	1.4681	73.5	1.4744
44.0	1.4552	54.0	1.4619	64.0	1.4685	74.0	1.4747
44.5	1.4555	54.5	1.4623	64.5	1.4688	74.5	1.4750
45.0	1.4558	55.0	1.4626	65.0	1.4691	75.0	1.4753
45.5	1.4562	55.5	1.4629	65.5	1.4694	75.5	1.4756
46.0	1.4565	56.0	1.4633	66.0	1.4697	76.0	1.4759
46.5	1.4569	56.5	1.4636	66.5	1.4700	76.5	1.4762
47.0	1.4572	57.0	1.4639	67.0	1.4704	77.0	1.4765
47.5	1.4576	57.5	1.4642	67.5	1.4707	77.5	1.4768
48.0	1.4579	58.0	1.4646	68.0	1.4710	78.0	1.4771
48.5	1.4583	58.5	1.4649	68.5	1.4713	78.5	1.4774
49.0	1.4586	59.0	1.4652	69.0	1.4717	79.0	1.4777
49.5	1.4590	59.5	1.4656	69.5	1.4720	79.5	1.4780

## MELTING POINT OF FATS AND FATTY ACIDS

*Wiley Method—Official*

12

## REAGENT

*Alcohol-water mixture.*—The sp. gr. should be the same as that of the fat to be examined. Prepare by boiling, separately, H<sub>2</sub>O and 95% alcohol for 10 min. to remove the gases that may be held in soln. While still hot pour the H<sub>2</sub>O into a test tube until it is almost half full. Nearly fill the test tube with the hot alcohol, pouring it down the side of the inclined tube to avoid too much mixing. If the alcohol is added after the H<sub>2</sub>O has cooled, air bubbles will make the mixture unfit for use.

13

## DETERMINATION

Allow the melted and filtered fat to fall a distance of 15–20 cm from a dropping tube upon a piece of ice or upon the surface of cold Hg. The disks thus formed should be 1–1.5 cm in diameter and should weigh about 200 mg. Remove the disks when solid, and allow to stand 2–3 hours in order to obtain the normal melting point.

Place a 30×3.5 cm test tube, containing the alcohol-water mixture, in a tall 35×10 cm beaker containing ice and H<sub>2</sub>O, and leave until the mixture is cold. Then drop a disk of fat into the tube. It will sink immediately to a point where the density of the alcohol-water mixture is exactly equivalent to its own. Lower an accurate thermometer, which can be read to 0.1°, into the test tube until the bulb is just above the disk. In order to secure an even temp. in all parts of the alcohol-water mixture around the disk, stir gently with the thermometer. Slowly heat the H<sub>2</sub>O in the beaker, constantly stirring it by means of an air blast or other suitable device.

When the temp. of the alcohol-water mixture rises to about 6° below the melting point of the fat, the disk of fat begins to shrivel and gradually rolls up into an irregu-

lar mass. Lower the thermometer until the fat particle is even with the center of the bulb. Rotate the thermometer bulb gently and so regulate the heat that about 10 min. is required for the last  $2^{\circ}$  increase in temp. As soon as the fat mass becomes spherical, read the thermometer. Remove the tube from the bath and again cool. Place in the bath a second tube containing the alcohol-water mixture. The test tube is of sufficiently low temp. to cool the bath to the desired point. After the first or preliminary determination, regulate the temp. of the bath so as to obtain a maximum of about  $1.5^{\circ}$  above the melting point of the fat under examination.

If the edge of the disk touches the sides of the tube, make a new determination. Run triplicate determinations. The second and third results should agree closely.

## 14

*Capillary Tube Method—Official*

Draw the melted fat or fatty acids into a thin-walled capillary tube. Use a column of fat 1–2 cm long, according to the length of the thermometer bulb. Seal one end of the tube and cool on ice 12–15 hours. Attach the capillary tube to the bulb of an accurate thermometer graduated to  $0.2^{\circ}$ , immerse in a large test tube of  $H_2O$  surrounded by a beaker of  $H_2O$ , and heat very slowly. An apparatus similar to that indicated in Fig. 37 may be used. Take as the melting point the temp. at which the substance becomes transparent.

## TITER TEST

*Alcoholic or Aqueous Sodium Hydroxide Method—Official*15 SPECIFICATIONS FOR TITER TEST THERMOMETERS<sup>7</sup>—OFFICIAL, FIRST ACTION

The original specification for the titer test thermometer is about twenty years old and on account of certain arbitrary limits in the specifications has always been a difficult and expensive thermometer to manufacture. It appears, furthermore, that it was originally designed to be read to  $1/10$  or  $1/5$  of a division, that is, to  $0.01^{\circ}$  or  $0.02^{\circ}$ , whereas, in practice, it is read to the nearest division, or perhaps occasionally to  $1/2$  division. The original specifications were difficult principally because it was desirable to keep the thermometer as short as possible, and this resulted in crowding the division marks so close together that reading was not easy. A slightly shorter, much more easily readable thermometer is obtained by subdividing the scale to  $0.2^{\circ}$  C. with a scale sufficiently open to make reading to  $1/2$  division,  $0.1^{\circ}$ , easy. This thermometer has been designed so as to cause no undue difficulties in manufacture, and at the same time to meet fully the requirements for accuracy in the titer test.

*Type.*—Etched stem, glass.

*Liquid.*—Mercury.

*Range and subdivision.*—Minus 2 to  $62^{\circ}$  in  $0.2^{\circ}$ , with expansion chamber at top.

*Total length.*—350–360 mm.

*Stem.*—Plain front, enamel back, suitable thermometer tubing. Diameter, 6 7 mm.

*Bulb.*—Corning normal or equally suitable thermometric glass. Diameter not less than 5.5 mm but not greater than that of the stem. Length, 20–30 mm.

*Distance to  $-2^{\circ}$  mark from bottom of bulb.*—45–60 mm.

*Distance to  $62^{\circ}$  mark from top of thermometer.*—20–50 mm.

*Length of unchanged capillary.*—Between top of bulb and the first graduation mark, 13 mm, and between the last graduation mark and the expansion chamber at the top, 10 mm.

*Top finish.*—Glass ring or knob.

*Filling above mercury.*—Nitrogen or other suitable gas.

*Graduation.*—All lines, figures, and letters to be clear cut and distinct. Each degree mark to be longer than the remaining lines. Graduations to be numbered at every 2° mark.

*Immersion.*—Total.

*Special marking. A.O.A.C. titer test.*—A serial number, and the manufacturer's name or trademark shall be etched upon the stem. The marking "0.2°C." shall be marked on the front of the stem above the scale.

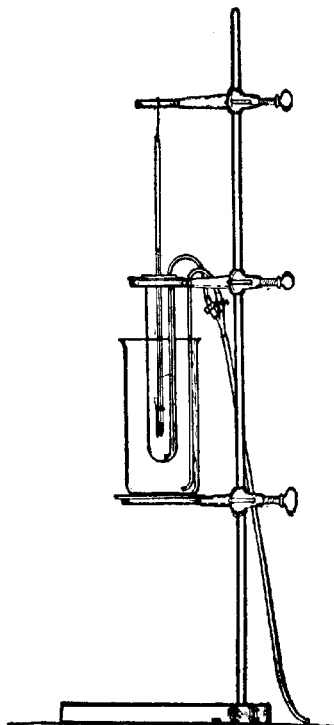


FIG. 37.—APPARATUS FOR THE DETERMINATION OF THE MELTING POINT

*Scale error.*—The error at any point on the scale, when the thermometer is standardized at total immersion, shall not exceed 0.2°C.

*Case.*—The thermometer shall be supplied in a suitable case on which shall appear the marking: *A.O.A.C. Titer Test, -2° to 62°C. in 0.2°.*

**NOTE:** For the purpose of interpreting these specifications the following definitions apply:

The total length is the over-all length of the finished instrument.

The diameter is that measured with a ring gage.

The length of the bulb is the distance from the bottom of the bulb to the beginning of the enamel backing.

The top of the thermometer is the top of the finished instrument.

## 16

## DETERMINATION

Saponify 75 g of the sample in a metal dish with 60 cc of 30% NaOH soln (36° Baumé) and 75 cc of 95% alcohol or 120 cc of  $H_2O$ . Evaporate to dryness over a very low flame or on an iron or asbestos plate, stirring constantly. Dissolve the dry soap in 1 liter of boiling  $H_2O$ , and if alcohol has been used boil for 40 min. to remove it, adding sufficient  $H_2O$  to replace that lost in boiling. Liberate the fatty acids by adding 100 cc of  $H_2SO_4$  (1+3) and boil until they form a clear, transparent layer. Wash the fatty acids with boiling  $H_2O$  until free from  $H_2SO_4$ , collect in a small beaker, and place on a steam bath until the  $H_2O$  has settled and the fatty acids are clear. Decant into a dry beaker, filter while hot, and dry 20 min. at 100°. When dried, cool the fatty acids to 15–20° above the expected titer and transfer to the titer tube, 25 by 100 mm (1 by 4 in.) and made of glass about 1 mm in thickness. Place in a 16 oz wide-mouthed bottle of clear glass, 70 by 150 mm (2.8 by 6 in.), fitted with a perforated cork so as to hold the tube rigidly when in position. Suspend the standard thermometer so that it can be used as a stirrer and stir the mass slowly until the Hg remains stationary for 30 seconds. Then allow the thermometer to hang quietly, with the bulb in the center of the mass, and observe the rise of the Hg column. The highest point to which it rises is regarded as the titer of the fatty acids.

Test the fatty acids for complete saponification as follows: Place 3 cc in a test tube and add 15 cc of 95% alcohol. Bring the mixture to a boil and add an equal volume of  $NH_4OH$  (1+2). A clear soln should result. Make the titer at about 20° for all fats having a titer above 30°, and at 10° below the titer for all other fats.

## 17

*Glycerol-Potassium Hydroxide Method—Official*

Heat 75 cc of glycerol KOH soln (25 g of KOH in 100 cc of U.S.P. glycerol) to 150° in an 800 cc beaker and add 50 cc of the oil or melted fat, previously filtered if necessary to remove foreign substances. Saponification often takes place almost immediately, but heating and frequent stirring should be continued for 15 min. Do not use a temp. much above 150°. When the saponification is complete, as indicated by the perfectly homogeneous soln, pour the soap into an 800 cc casserole containing about 500 cc of nearly boiling  $H_2O$ ; add carefully 50 cc of  $H_2SO_4$  (1+3); and heat the soln, with frequent stirring, until the layer of fatty acids separates out perfectly clear. Transfer the fatty acids to a tall separatory funnel, wash 3 or 4 times with boiling  $H_2O$  to remove all mineral acids, draw the fatty acids off into a small beaker, and allow to stand on a steam bath until the  $H_2O$  has settled out and the acids are clear. Filter into a dry beaker and heat to 150° on a thin asbestos plate, stirring continually with the thermometer; transfer to a titer tube, fill it to within 2.5 cm of the top, and take the titer as directed under 16.

## IODINE ABSORPTION NUMBER

(All reports should specify the method used.)

*Hanus Method—Official*

## 18

## REAGENTS

(a) *Hanus iodine soln.*—Dissolve 13.2 g of pure I in 1 liter of glacial acetic acid (99.5%) that shows no reduction with dichromate and  $H_2SO_4$ . Add enough Br to

double the halogen content as determined by titration (about 3 cc). The I may be dissolved by heating, but the soln should be cold when the Br is added.

A convenient procedure for preparing the Hanus I soln is as follows: Measure 825 cc of acetic acid that has shown no reduction by the dichromate test and dissolve in it with the aid of heat 13.615 g of I. Cool and titrate 25 cc of this soln with the 0.1 N  $\text{Na}_2\text{S}_2\text{O}_3$ . Measure another portion of 200 cc of the acetic acid and add 3 cc of Br. To 5 cc of this soln add 10 cc of the 15% KI soln, and titrate with the 0.1 N  $\text{Na}_2\text{S}_2\text{O}_3$ . Calculate the quantity of the Br soln required to double the halogen content of the remaining 800 cc of I soln as follows:

$$A = \frac{B}{C}, \text{ in which}$$

$A$  = cc of Br soln required;

$B$  =  $800 \times$  the thiosulfate equivalent of 1 cc of I soln; and

$C$  = the thiosulfate equivalent of 1 cc of Br soln.

EXAMPLE: 136.15 g of I is dissolved in 8250 cc of acetic acid, and 30 cc of Br is dissolved in 2000 cc of acetic acid. Titrating 50 cc of the I soln against the standard thiosulfate shows that 1 cc of the I soln = 1.1 cc of the thiosulfate (0.0165 g of I). Titrating 5 cc of the Br soln shows that 1 cc of the Br soln = 4.6 cc of the thiosulfate. Then the quantity of Br soln required to double the halogen content of the remain-

ing 8200 cc of I soln =  $\frac{8200 \times 1.1}{4.6}$ , or 1961 cc. Upon mixing the two solns in this

proportion, there is obtained a total volume of 10161 cc, containing 135.3 g of I. In order to reduce this soln to the proper strength (13.2 g of I per liter),  $10,161 \times 13.2 =$

134.1;  $135.3 - 134.1 = 1.2$  g of I present in excess, or  $\frac{1.2 \times 1000}{13.2} = 91$  cc of acetic

acid, which must be added.

(b) *Sodium thiosulfate soln.*—0.1 N. Prepare a soln containing 24.82 g of  $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$  in freshly boiled and cooled  $\text{H}_2\text{O}$  and dilute to 1 liter. Standardize this soln as follows: Place in a glass-stoppered flask 20 cc of the 0.1 N  $\text{K}_2\text{Cr}_2\text{O}_7$  and 10 cc of the 15% KI soln. Add 5 cc of HCl. Dilute with 100 cc of freshly boiled and cooled  $\text{H}_2\text{O}$  and allow the 0.1 N  $\text{Na}_2\text{S}_2\text{O}_3$  to flow slowly into the flask until the yellow color of the liquid has almost disappeared; add a few drops of starch indicator, VI, 3(e); and with constant shaking, continue to add the 0.1 N  $\text{Na}_2\text{S}_2\text{O}_3$  until the blue color just disappears.

(c) *Potassium dichromate.*—0.1 N. Dissolve 4.903 g of  $\text{K}_2\text{Cr}_2\text{O}_7$  in  $\text{H}_2\text{O}$  and dilute to 1 liter. Check the strength of this soln against pure Fe.

## 19

## DETERMINATION

Weigh about 0.500 g of fat, or 0.250 g of oil (0.100–0.200 g in the case of drying oils that have a very high absorbent power), into a 500 cc glass-stoppered flask or bottle. Dissolve the fat, or oil, in 10 cc of  $\text{CHCl}_3$ . Add 25 cc of the Hanus I soln and allow to stand for 30 min., shaking occasionally. (This time must be adhered to closely in order to obtain accurate results. The excess of I should be at least 60% of the quantity added.) Add 10 cc of 15% KI soln, shake thoroly, and then add 100 cc of freshly boiled and cooled  $\text{H}_2\text{O}$ , washing down any free I that may be found on the stopper. Titrate the I with the 0.1 N  $\text{Na}_2\text{S}_2\text{O}_3$ , adding it gradually, with con-



stant shaking, until the yellow color of the soln has almost disappeared. Add a few drops of starch indicator, VI, 3(e), and continue the titration until the blue color has entirely disappeared. Toward the end of the titration, stopper the bottle and shake violently, so that any I remaining in soln in the  $\text{CHCl}_3$  may be taken up by the KI soln. Conduct two blank determinations along with that on the sample. The number of cc of the 0.1  $N$   $\text{Na}_2\text{S}_2\text{O}_3$  required by the blank less the quantity used in the determination gives the thiosulfate equivalent of the I absorbed by the fat or oil. Calculate the percentage by weight of I absorbed and report as the I number (Hanus method).

*Wijs Method—Official*

## 20

## REAGENTS

*Wijs iodine soln.*—Dissolve 13 g of resublimed I in 1 liter of glacial acetic acid (99.5%) and pass in washed and dried Cl gas until the original thiosulfate titration of the soln is not quite doubled. Use not more than a slight excess of I and no excess of Cl. Preserve in a glass-stoppered amber bottle sealed with paraffin until ready for use. Do not use Wijs soln that is more than 30 days old. Because of its unstable character  $\text{ICl}_3$  should not be used for preparation of the I soln.<sup>8</sup>

The other reagents and solns used are described under 18.

## 21

## DETERMINATION

Weigh 0.10–0.50 g (depending on the I number) of the melted and filtered sample into a clean, dry, 16 oz, glass-stoppered bottle containing 15–20 cc of  $\text{CCl}_4$  or  $\text{CHCl}_3$ . With a pipet add 25 cc of the I soln, allowing the pipet to drain for a definite time. The excess of I should be from 50–60% of the quantity added, that is, from 100–150% of the quantity absorbed. Moisten the stopper with the 15% KI soln to prevent loss of I or Cl but guard against the use of a quantity sufficient to run down inside the bottle. Let the bottle stand in a dark place for 30 min. at a uniform temp. At the end of that time add 20 cc of 15% KI soln and 100 cc of recently boiled and cooled  $\text{H}_2\text{O}$ . Titrate the I with the 0.1  $N$   $\text{Na}_2\text{S}_2\text{O}_3$  soln, adding the latter gradually and with constant shaking until the yellow color of the soln has almost disappeared. Add a few drops of starch indicator, VI, 3(e), and continue the titration until the blue color has entirely disappeared. Toward the end of the reaction stopper the bottle and shake violently so that any I remaining in soln in the  $\text{CCl}_4$  or  $\text{CHCl}_3$  may be taken up by the KI soln. Conduct two determinations on blanks, run in the same manner as the sample, but without any fat. Slight variations in temp. affect quite appreciably the titer of the I soln as acetic acid has a high coefficient of expansion. It is essential, therefore, that the blanks and determinations on the sample be made at the same time. The number of cc of standard thiosulfate soln required by the blank less the quantity used in the determination gives the thiosulfate equivalent of the I absorbed by the sample taken. Calculate the percentage by weight of I absorbed and report as the I number (Wijs method).

**SAPONIFICATION NUMBER (KOETTSTORFER NUMBER)—OFFICIAL**

## 22

## REAGENT

*Alcoholic potassium hydroxide soln.*<sup>8</sup>—(1) Reflux 1.2 liters of 95% alcohol for 30 min. in a distilling flask with 10 g of KOH and 6 g of granulated Al (or foil). Distil, and collect 1 liter after discarding the first 50 cc. Dissolve 40 g of high-grade KOH in this liter of alcohol. Keep soln in a glass-stoppered bottle. Or (2) crush 40 g of

high-grade KOH in a 7 or 8 in. mortar. Add 45 g of granulated CaO and grind mixture to a powder. From a liter of 95% alcohol add 100 cc to the mortar and transfer to a flask, rinsing the mortar with several more portions. Add the remainder of the alcohol to the flask, shake the mixture for at least 5 min., and then invert a beaker over the neck of the flask. Repeat the shaking several times during the day. Next morning filter the soln into a clean, dry, glass-stoppered bottle.

## 23

## DETERMINATION

Weigh accurately about 5 g of the filtered sample into a 250–300 cc Erlenmeyer flask. Pipet 50 cc of the alcoholic KOH soln into the flask, allowing the pipet to drain for a definite time. Connect the flask with an air condenser and boil until the fat is completely saponified (about 30 min.). Cool, and titrate with 0.5 N HCl, II, 19(a), using phenolphthalein indicator. Conduct a blank determination along with that on the sample, using the same pipet for measuring the KOH soln and draining for the same length of time. Subtract the number of cc of 0.5 N HCl obtained in the determination on the sample from the number obtained on the blank to obtain the cc of 0.5 N HCl equivalent to the KOH used in the saponification of the sample taken. Calculate and report as saponification number (mg of KOH required to saponify 1 g of fat).

## 24

## SOLUBLE ACIDS—OFFICIAL

Place the flask used under 23 and its contents on a water bath and evaporate the alcohol. Add that quantity of 0.5 N HCl which is equivalent to the quantity of KOH used for the saponification of the sample under 23 and 1 cc more (quantity of 0.5 N HCl to be added = titration for blank – titration for sample + 1 cc), and place the flask on the steam bath until the separated fatty acids form a clear layer on the upper surface of the liquid. Fill the flask to the neck with hot H<sub>2</sub>O and cool the contents in ice H<sub>2</sub>O until the cake of fatty acids is thoroly hardened. Pour the liquid contents of the flask thru a filter into a liter flask, refill the flask with hot H<sub>2</sub>O, and set on the steam bath until the fatty acids collect at the surface. Cool by immersing in ice H<sub>2</sub>O and again filter the liquid into the liter flask. Repeat this treatment with hot H<sub>2</sub>O 3 times, cooling and collecting the washings in the liter flask after each treatment. Titrate the combined washings with 0.1 N alkali, using phenolphthalein indicator. Subtract 5 (corresponding to the excess of 1 cc of 0.5 N acid) from the number of cc of 0.1 N alkali used and multiply by 0.0088 to obtain the weight of soluble acids as butyric acid. Calculate the percentage of soluble acids.

## 25

## INSOLUBLE ACIDS (HEHNER NUMBER)—OFFICIAL

Allow the flask containing the cake of insoluble fatty acids from 24 and the paper thru which the soluble fatty acids have been filtered to drain and dry for 12 hours. Transfer the cake, together with as much of the fatty acids as can be removed from the filter paper, to a weighed, wide-mouthed beaker flask. Then place the funnel containing the filter in the neck of the flask and wash the paper thoroly with hot absolute alcohol. Remove the funnel, evaporate the alcohol, dry for 2 hours at 100°, cool in a desiccator, and weigh. Again dry for 2 hours, cool, and weigh. If there is any considerable decrease in weight, re-heat for 2 hours, cool, and weigh again. Calculate the percentage of insoluble fatty acids.

SOLUBLE VOLATILE ACIDS (REICHERT-MEISSL AND POLENSKE VALUES)<sup>18</sup>—OFFICIAL

26

## REAGENTS

(a) *Sodium hydroxide soln.*—(1+1). Protect the soln from contact with CO<sub>2</sub>. Allow the soln to settle and use only the clear liquid.

(b) *Pumice stone.*—Heat small pieces to a white heat, plunge into H<sub>2</sub>O, and keep there until used.

(c) *Glycerol-soda soln.*—Add 20 cc of the 1+1 NaOH soln to 180 cc of pure concentrated glycerol.

27

## DETERMINATION

Weigh accurately 5 g of the sample to be tested into a clean, dry, 300 cc flask; add 20 cc of the glycerol-soda soln and heat over a flame or asbestos plate until complete saponification occurs, as shown by the mixture becoming perfectly clear. If foaming occurs, shake the flask gently. Add 135 cc of recently boiled H<sub>2</sub>O, drop by drop at first to prevent foaming, then add 6 cc of H<sub>2</sub>SO<sub>4</sub> (1+4) and a few fragments of pumice stone. Distil without previously melting the fatty acids, using an apparatus of the approximate dimensions illustrated in the diagram (Fig. 38). Rest the flask on a piece of asbestos board having a hole 5 cm in diameter in the center, and so regulate the flame as to collect 110 cc of the distillate in as near 30 min. as possible and to allow the distillate to drip into the receiving flask at a temp. not higher than 18–20°.

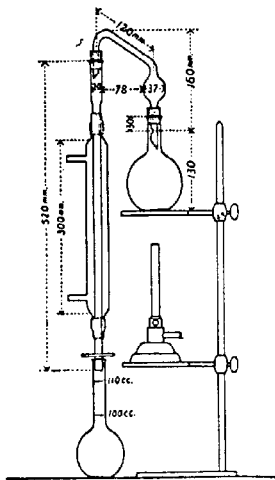


FIG. 38.—APPARATUS FOR THE DETERMINATION OF THE POLENSKE NUMBER

When the distillation is complete, substitute for the receiving flask a 25 cc cylinder to collect any drops that may fall after the flame has been removed. Mix without violent shaking, immerse the flask containing the distillate almost completely in

H<sub>2</sub>O at 15° for 15 min., filter the 110 cc of distillate thru a dry filter paper 9 cm in diameter, and titrate 100 cc with the standard NaOH soln, using phenolphthalein (1% alcoholic soln) as an indicator. The pink color should remain unchanged for 2 or 3 min. The Reichert-Meissl value is the number of cc of 0.1 N NaOH soln used times 1.1, after this result is corrected for the figure obtained in a blank determination.

Remove the remainder of the soluble acids from the insoluble acids upon the filter paper by washing with 3 successive 15 cc portions of H<sub>2</sub>O, previously passed thru the condenser, the 25 cc cylinder, and the 110 cc receiving flask. Then dissolve the insoluble acids by passing successive 15 cc portions of neutral alcohol, 95% by volume, thru the filter paper, each portion having previously passed thru the condenser, the 25 cc cylinder, and the 110 cc receiving flask. Titrate the combined alcoholic washings with the standard NaOH soln, using the phenolphthalein as indicator. The Polenske value equals the number of cc of alkali soln required for the titration.

NOTE.—Unless these directions are followed in every detail as described, satisfactory results cannot be obtained.

## 28

KIRSCHNER VALUE<sup>11</sup>—OFFICIAL

To 100 cc of the Reichert-Meissl distillate, in a 200 cc Erlenmeyer flask, add 6 drops of phenolphthalein soln and titrate to a very faint pink with a 0.1 N Ba(OH)<sub>2</sub> soln. Add 0.3 g of finely powdered Ag<sub>2</sub>SO<sub>4</sub>. During the next hour shake the mixture frequently, then filter and transfer 100 cc of the filtrate to a 300 cc flask. Add 10 cc of H<sub>2</sub>SO<sub>4</sub> (1+40), 35 cc of H<sub>2</sub>O, and a piece of Al wire or several small pieces of pumice stone, 26(b). Distil 110 cc in about 20 min., using the Polenske apparatus, 27. Titrate 100 cc of the distillate with 0.1 N Ba(OH)<sub>2</sub> soln, make a blank determination, and after correcting the number of cc of alkali used, calculate the Kirschner

value according to the following formula:  $K = \frac{A \times 121 (100 + B)}{10,000}$ , in which  $A$  = the

corrected Kirschner titration and  $B$  = the number of cc of standard alkali soln to neutralize the 100 cc Reichert-Meissl distillate.

Butter fat gives Kirschner values from 19 to 26, coconut oil gives an average of 1.9, and palm kernel oil, 1.0, whereas the majority of other fats and oils give values from 0.1 to 0.2.

## SATURATED AND UNSATURATED FATTY ACIDS

## 29

Lead Salt-Ether Method<sup>12</sup>—Official

(Not applicable to fats and oils that contain erucic, elaeostearic, chaulmoogric, hydrocarpic or similar acids; to hydrogenated products that contain notable quantities of iso-oleic acid; nor to coconut or palm kernel oils that contain notable quantities of the lower fatty acids which give ether-soluble Pb salts.)

Weigh accurately 10 or 20 g of the sample into a 200 cc Erlenmeyer flask. Add 30 cc of alcohol and 8 cc of KOH soln (1+1). Mix thoroly and heat on the steam bath for about 30 min. Add a slight excess of acetic acid, using phenolphthalein as an indicator, and then add a sufficient quantity of 15% KOH soln while rotating the flask to produce a distinct pink color. Heat to boiling in a liter flask 60 cc (120 cc for 20 g of sample) of 20% Pb acetate soln and the same quantity of H<sub>2</sub>O. Add the neutralized soap soln cautiously to avoid any loss, rinsing the saponification flask

with 5 cc of alcohol, then with small volumes of hot  $H_2O$ . Boil the mixture gently for about 5 min., shake thoroly, and cool under running  $H_2O$ , rotating the flask to cause all the precipitated Pb soaps to adhere to the sides and bottom of the flask. When the mixture is cold pour off the aqueous soln into a large beaker in order to examine the soln for particles of Pb soap. (Usually the soln is slightly turbid owing to some basic Pb acetate, and no particles or globules of Pb soap are seen.) Wash the flask and Pb soap twice with cold  $H_2O$  and allow the flask to drain for 10 min. Remove the last drops of  $H_2O$  by means of a thin roll of filter paper held by forceps, being careful to press the paper only lightly against the precipitate. Add about 120 cc of ether and shake by rotating the flask for about 5 min.

Connect the flask with a reflux condenser and boil the contents gently until the Pb soap is completely disintegrated or dissolved. Remove the flask and rinse down the sides with sufficient ether to make the final volume about 150 cc. Invert a close fitting beaker over the neck of the flask and place in an ice box for at least 15 hours. Place a 7 cm ordinary filter paper in a Büchner funnel of 7.5 cm diameter, turn on full suction, and fit a hardened filter paper cut to 8 cm in diameter as snugly as possible to the sides of the funnel. Decant the ether soln from the separated Pb soaps, using only sufficient suction to draw the ether thru the filter. (Too much suction causes the ether to evaporate so rapidly that the filter may become clogged with the separated unsaturated acids, Pb soaps, or ice.)

Transfer the precipitate to the filter by rinsing the flask with small portions of ether. During filtration keep the funnel covered as much of the time as possible to prevent the evaporation of the ether. If at any time filtration proceeds so fast as to cause the mass of Pb soap to crack, close the cracks by pressing with a small spoon or spatula; otherwise the precipitate cannot be properly washed. Rinse the spoon free from the precipitate and wash the precipitate 8 or 10 times with ether, finally allowing the suction to continue until the precipitate cracks into numerous pieces. Without delay, separate with a spoon as much of the precipitate as possible and transfer it without loss to a 500 cc separatory funnel containing about 50 cc of ether, washing off any precipitate adhering to the spoon and neck of the separatory funnel with ether. Transfer the filter paper to the liter flask. Shake the contents of the separatory funnel thoroly to disintegrate the lumps of Pb salt and allow to stand for about 20 min. Add 20 cc of HCl previously diluted with 10 cc of  $H_2O$  and shake thoroly for 2 min. to decompose all the Pb soap. Add 5–10 cc of HCl (2+1) to the liter flask containing the filter paper, shake thoroly to decompose any precipitate adhering to the flask and filter; then wash into the separatory funnel with small alternate portions of ether and  $H_2O$  until all the fatty acids and  $PbCl_2$  are removed from the flask. Again shake the contents of the separatory funnel with a rotary motion and allow to stand for 10 min. Withdraw the lower aqueous soln slowly, taking precautions not to remove any emulsion or undecomposed Pb soap. When Pb soap is present (shown in the form of lumps that float on top of the aqueous soln), add 10 cc of HCl and shake again; then add about 20 cc of  $H_2O$ , shake, and allow the mixture to stand until the layers have separated. Withdraw the aqueous soln and wash the ether with successive 25 cc portions of  $H_2O$  until the washings are free from HCl. Dehydrate the ether with about 2 g of anhydrous  $Na_2SO_4$  and transfer the soln to a weighed 300 cc Erlenmeyer flask. Rinse the separatory funnel and  $Na_2SO_4$  with several small portions of ether to remove all the fatty acids, taking care not to allow any of the  $Na_2SO_4$  to fall into the weighed flask. Distil the ether, avoiding any loss of the fatty acids, and heat in an oven at about  $110^\circ$  until the weight is constant. Obtain the weight of the saturated acids and save them for later investigation.

Transfer the ether soln of the soluble Pb soaps to a 500 cc or a 1000 cc separatory funnel, rinsing the Büchner funnel and filter flask with small quantities of ether. Add a mixture of 30 cc of HCl and 75 cc of H<sub>2</sub>O and shake with a rotary motion for 2 min. After allowing the mixture to stand for 10 min., slowly withdraw the aqueous soln into a beaker. If drops of the ether soln are entrapped by the PbCl<sub>2</sub> precipitate and are removed with it, decant the soln from the precipitated PbCl<sub>2</sub> that has settled into the separatory funnel. Rinse the beaker and precipitate with small quantities of ether, adding the washings to the separatory funnel. Rotate the contents of the separatory funnel and allow to stand for 10 min. Withdraw the aqueous soln and wash the ether with successive 50 cc portions of H<sub>2</sub>O until the HCl is removed. Transfer the ether soln to a 300 cc weighed Erlenmeyer flask. Distil the ether and place the flask in an oven heated to about 110° for about 1 hour, while passing a stream of CO<sub>2</sub> into the flask to prevent oxidation of the unsaturated acids. Cool in an atmosphere of CO<sub>2</sub>. When cold, remove the CO<sub>2</sub> and weigh. Repeat this treatment until a constant weight is obtained.

Determine in duplicate the I numbers of both the saturated and unsaturated acid fractions. (The I number of the saturated acid fraction is due to the presence of some unsaturated acid.)

To correct for the unsaturated acids present in the fraction of saturated acids use the following formula:

$$\frac{\text{I No. of saturated acid fraction}}{\text{I No. of unsaturated acid fraction}} \times 100 = A \quad (\text{percentage of unsaturated acids in saturated acid fraction}).$$

Obtain the correct value by means of the formula  $\frac{A \times B}{100}$ , in which *B* is the percentage of the impure saturated acids (as found by analysis). Subtract this correction from the percentage of impure saturated acids and add it to the percentage of unsaturated acids actually determined.

## 30

## FREE FATTY ACIDS—OFFICIAL

Weigh 20 g of fat, or oil, into a flask; add 50 cc of 95% alcohol that has been neutralized with 0.1 *N* NaOH soln, using phenolphthalein indicator; and heat to boiling. Shake the flask thoroly in order to dissolve the free fatty acids as completely as possible. Titrate with 0.1 *N* NaOH or KOH, shaking thoroly until the pink color persists after vigorous shaking. Express the results as percentage of oleic acid, as acid degree (cc of 1 *N* alkali required to neutralize the free acids in 100 g of oil or fat), or as acid value (mg of KOH required to saturate the free acids in 1 g of fat or oil). 1 cc of 0.1 *N* alkali = 0.0282 g of oleic acid.

ACETYL VALUE<sup>12</sup>—OFFICIAL

## 31

*Acetylation*

Boil 50 cc of the sample with 50 cc of freshly distilled acetic anhydride under a reflux condenser for 2 hours. Pour the mixture into 500 cc of H<sub>2</sub>O in a beaker and boil for 15 min. while bubbling a stream of air or of CO<sub>2</sub> thru the soln to prevent bumping. Siphon off the H<sub>2</sub>O, add 500 cc more of H<sub>2</sub>O, and boil again for 15 min. Repeat the siphonation and boil for 15 min. with a third 500 cc portion of H<sub>2</sub>O. Allow the mixture to cool and separate the aqueous layer, which should be neutral to litmus. Transfer the acetylated oil to a separatory funnel and wash with two 200 cc portions of warm H<sub>2</sub>O. Separate as much of the H<sub>2</sub>O as possible, add 5 g of anhydrous Na<sub>2</sub>SO<sub>4</sub> to the acetylated oil, and let stand for 1 hour, agitating occasionally

to assist the drying. Filter thru a dry folded filter, preferably in an oven heated to 100–110°, and keep the filtered oil in the oven until the oil is completely dry. The acetylated product should be a clear, brilliant oil.

## 32

*Saponification*

Weigh accurately 2–2.5 g each of the acetylated oil and of the untreated oil into separate 250 cc Erlenmeyer flasks. Add to each flask exactly 25 cc of alcoholic KOH soln, 22, and reflux for 1 hour. Titrate the warm solns with 0.5 *N* HCl, using phenolphthalein as indicator. Titrate in the same way two 25 cc portions of the alcoholic KOH soln. From the mean of these two results, which should be in very close agreement, deduct the volume of the standard HCl required for the titration of the acetylated and of the untreated oil and from the results so obtained calculate the saponification number (mg of KOH required to saponify 1 g of product) of each. Calculate the acetyl value by means of the following formula:

$$A = \frac{S' - S}{1 - 0.00075S}, \text{ in which}$$

*A* = acetyl value;

*S* = saponification number of oil; and

*S'* = saponification number of the acetylated oil.

## CHOLESTEROL AND PHYTOSTEROL IN MIXTURES OF ANIMAL AND VEGETABLE FATS

## 33

*Alcohol Extraction Method*<sup>14</sup>—*Tentative*

Introduce 200–300 g of the melted fat into a flat-bottomed liter flask. Close the neck of the flask with a 3 holed stopper and insert thru these holes: (1) A reflux condenser; (2) a right-angled glass tube, one arm of which reaches to a point 6 mm above the surface of the melted fat, the other being closed a short distance from the flask by means of a short piece of rubber tubing and a pinch-cock; (3) a glass tube bent so that one arm reaches down to the bottom of the flask and the other serves as a delivery tube for a 700 cc round-bottomed flask containing 500 cc of 95% alcohol.

Place the flasks containing the melted fat and the alcohol on a steam bath and heat so that the alcohol vapor passes thru the melted fat in the liter flask and is condensed in the reflux condenser, finally collecting in a layer over the melted fat. After all the alcohol has passed in this manner into the flask containing the fat, disconnect the flask from which the alcohol has been distilled and attach a tube to the short piece of rubber tubing attached to the right-angled glass tube, see (2) above, and siphon the alcohol layer back into the alcohol distillation flask. Reconnect as at first and again distil the alcohol as in the first operation. When all the alcohol has been distilled, siphon it again into the distillation flask and extract in the same manner a third time.

Discard the fat and retain the alcohol, which now contains practically all the cholesterol and phytosterol originally present in the fat. Concentrate the alcoholic soln to about 250 cc, and to the boiling liquid add 20 cc of KOH soln (1+1). Boil for 10 min. to insure complete saponification of the fat, cool to room temp., and pour into a large separatory funnel containing 500 cc of warm ether. Shake to insure thorough mixing and add 500 cc of H<sub>2</sub>O. Rotate the funnel gently to avoid the formation of extremely stubborn emulsions, but mix the H<sub>2</sub>O thoroughly with the alcohol-ether-soap soln. A clear, sharp separation takes place at once. Draw off the soap soln and wash the ether layer with 300 cc of H<sub>2</sub>O, avoiding shaking. Repeat the washing of the

ether soln with small quantities of  $H_2O$  until all the soap is removed. Transfer the ether layer to a flask and distil the ether until the volume of liquid remaining in the flask measures about 25 cc. Transfer this residue to a tall 50 cc beaker and continue the evaporation until all the ether is driven off and the residue is perfectly dry. If desired, a weighed beaker may be used and the weight of the unsaponifiable matter determined at this point.

Add 3-5 cc of acetic anhydride to the residue in the beaker, cover the beaker with a watch-glass, and heat to boiling over a free flame. After boiling for a few seconds, remove the beaker from the flame, cool, and add 35 cc of alcohol, 60% by volume. Mix the contents of the beaker thoroly, filter off the alcoholic soln, and wash the precipitate with the 60% alcohol. Dissolve the precipitate on the filter with a stream of hot alcohol, 80% by volume, and wash the insoluble portion well with the 80% alcohol. Acetates of cholesterol and phytosterol are dissolved, while the greater portion of the impurities present (including paraffin and paraffin oil) remains behind on the filter. Cool the combined filtrate and washings to a temp. of  $10-12^\circ$  and allow to stand at that temp. for 2-3 hours. During this time the acetates of cholesterol and phytosterol crystallize from the soln. Collect the crystals upon a filter, wash with cold alcohol, 80% by volume, and then dissolve in a minimum quantity of hot absolute alcohol. Collect the alcoholic soln of the acetates in a small glass evaporating dish, add 2 or 3 drops of  $H_2O$  to the soln, and heat if not perfectly clear. Allow the alcohol to evaporate spontaneously, stirring the contents of the dish occasionally to mix the deposit of crystals that form upon the edges with the main body of the liquid. As soon as a good deposit of crystals has formed, collect them upon a hardened filter; wash twice with cold alcohol, 90% by volume; and dry by suction, drying finally at  $100^\circ$  for 30 min. Determine the melting point in the apparatus shown in the figure under 15, using  $H_2SO_4$  in the outer beaker and glycerol in the inner tube.

The melting point of the first crop of crystals usually gives definite information as to the presence or absence of phytosterol, but the conclusion indicated should be confirmed by recrystallizing the crystals from absolute alcohol and again determining the melting point. If the crystals are pure cholesteryl acetate, the melting point of the second crop should agree closely with that of the first. If phytosteryl acetate is present, however, a higher melting point will be noted, as phytosteryl acetate is less soluble in alcohol than cholesteryl acetate. The melting point of cholesteryl acetate is  $114^\circ$ ; that of phytosteryl acetate,  $125-137^\circ$ .

#### 34

#### *Digitonin Method<sup>12</sup>—Tentative*

Shake vigorously 50 g of the oil, or fat, for 15 min. in a separatory funnel with 20 cc of a 1% soln of digitonin in 95% alcohol. Allow the mixture to stand for a time until the emulsion separates. The lower or fat layer should be quite clear while the alcohol layer contains a bulky, flocculent precipitate. Draw off as much of the fat as possible, avoiding any loss of the precipitate. Add 100 cc of ether to the alcohol layer and filter the mixture. After drying in the air wash the precipitate with ether until free from fat, transfer to a tall 50 cc beaker, and add 2-3 cc of acetic anhydride. Cover the beaker with a watch-glass. Then boil slowly over a low flame for 30 min. After cooling, add 30-35 cc of alcohol, 60% by volume, and mix the contents of the beaker thoroly. Filter the alcohol soln. Wash the precipitate with the 60% alcohol, then dissolve on the filter with a stream of hot alcohol, 80% by volume, from a wash bottle, and set aside the filtrate in a cool place ( $10^\circ$  or below). After the acetates have crystallized out of this soln filter them off, recrystallize from absolute alcohol, dry, and determine the melting point of each crop of crystals as directed under 33.



## UNSAAPONIFIABLE RESIDUE

*F. A. C. Method<sup>16</sup>—Official*

35

## REAGENT

*Petroleum ether.*—Redistil below 75°. Make a blank determination by evaporating 350 cc of the reagent with about 0.25 g of stearine or other hard fat (previously brought to constant weight by heating) and drying as in the actual determination. The blank must not exceed a few mg.

36

## APPARATUS

*Extraction cylinder.*—Glass-stoppered, graduated at 40 cc, 80 cc, and 130 cc, and of the following dimensions: diameter about 1½ in., height about 12 in.

37

## DETERMINATION

Weigh 5 g ( $\pm 0.020$  g) of the prepared sample into a 200 cc Erlenmeyer flask, add 30 cc of redistilled approximately 95% alcohol (by volume) and 5 cc of 50% aqueous KOH, and boil the mixture for 1 hour under a reflux condenser. Transfer to the extraction cylinder and wash to the 40 cc mark with redistilled 95% alcohol. Complete the transfer, first with warm, then with cold H<sub>2</sub>O, until the total volume is 80 cc. Rinse the flask with 50 cc of petroleum ether and add the rinsings to the contents of the cylinder previously cooled to room temp. Shake as vigorously as possible for 1 min. and allow to settle until both layers are clear, when the volume of the upper layer should be about 40 cc. Draw off the petroleum ether layer as closely as possible by means of a slender glass siphon into a separatory funnel of 500 cc capacity. Repeat the extraction at least 6 more times, using 50 cc of petroleum ether for each extraction. Wash the combined extracts in the separatory funnel three times with 25 cc portions of 10% alcohol by volume, shaking vigorously each time. Transfer the petroleum ether extract to a weighed Erlenmeyer flask and distil; or, if desired, evaporate the petroleum ether on a steam bath in a current of air. Heat the flask with residue until a constant weight is obtained in an oven at a uniform temp. not less than 100° nor more than 110°. (A vacuum oven may be used at a corresponding temp., which depends upon the pressure used in it. It is important to displace with air any residue vapors of petroleum ether remaining in the flask after heating and before it is weighed.) Deduct any blank from the weight before calculating unsaponifiable matter. Test the final residue for solubility in 50 cc petroleum ether at room temp. Filter, and wash free from the insoluble residue, if any. Evaporate and dry in the same manner as before.

## RESIN OIL

38

*Qualitative Test—Tentative*

Polarize the pure oil, or a definite dilution, with petroleum ether in a 200 mm tube. Resin oil has a polarization in a 200 mm tube of from +30° to +40° on the sugar scale (Schmidt and Haensch), while most oils<sup>17</sup> read between +1° and -1°.

## COTTONSEED OIL

39

*Halphen Test<sup>18</sup>—Official*

Mix CS<sub>2</sub> containing 1% of S in soln with an equal volume of amyl alcohol. Mix equal volumes of this reagent and the sample under examination and heat in a bath of boiling, saturated brine for 1-2 hours. In the presence of as little as 1% cottonseed

oil, a pronounced characteristic red or orange-red color is produced. The depth of color is proportional, to a certain extent, to the quantity of cottonseed oil present, and by making comparative tests with known mixtures of cottonseed oil an approximation of the quantity present can be obtained.

Different oils react with different intensities. Oils that have been heated to 200–210°<sup>19</sup> react with greatly diminished intensity. Heating for 10 min. at 250° renders cottonseed oil incapable of giving the reaction.<sup>20</sup> The fat of animals fed on cottonseed meal or other cottonseed products may give a positive reaction by this test.

## 40

PEANUT OIL.<sup>21</sup>—OFFICIAL

Weigh 20 g of the oil into an Erlenmeyer flask. Saponify with alcoholic KOH soln, 22; neutralize exactly with acetic acid (1+3), using phenolphthalein indicator; and wash into an 800–1000 cc flask containing a boiling mixture of 100 cc of H<sub>2</sub>O and 120 cc of 20% Pb acetate soln. Boil for a min. and then cool the precipitated soap by immersing the flask in H<sub>2</sub>O, occasionally giving it a whirling motion to cause the soap to stick to the sides of the flask. After the flask has cooled, decant the H<sub>2</sub>O and excess of Pb acetate soln and wash the Pb soap with cold H<sub>2</sub>O and alcohol, 90% by volume. Add 200 cc of ether, cork, and allow to stand until the soap is disintegrated; heat on a water bath, using a reflux condenser, and boil for about 5 min.<sup>22</sup> In the case of oils, most of the soap will be dissolved, while in lards, which contain much stearin, part of the soap will be left undissolved. Cool the ether soln of soap to 15–17° and allow to stand until all the insoluble soaps have separated out (about 12 hours).

Filter upon a Büchner funnel and thoroly wash the insoluble Pb soaps with ether. Wash the ether-insoluble Pb soaps into a separatory funnel by means of a jet of ether, alternating at the end of the operation if a little of the soap sticks to the paper with HCl (1+3). Add sufficient HCl (1+3) so that the total volume of the acid amounts to about 200 cc and enough ether to make its total volume 150–200 cc and shake vigorously for several min. Allow the layers to separate, run off the acid layer, and wash the ether once with 100 cc of the dilute HCl and then with several portions of H<sub>2</sub>O until the H<sub>2</sub>O washings are no longer acid to methyl orange. If a few undecomposed lumps of Pb soap remain (indicated by solid particles remaining after the third washing with H<sub>2</sub>O), break these up by running off almost all the water layer and then add a little HCl; shake, and continue the washing with H<sub>2</sub>O as before. Distil the ether from the soln of insoluble fatty acids and dry the latter in the flask by adding a little absolute alcohol and evaporating on a steam bath. Dissolve the dry fatty acids by warming with 100 cc of 90% alcohol by volume. Cool slowly to 15°, shaking to aid crystallization. Allow to stand at 15° for 30 min. In the presence of peanut oil, crystals of arachidic acid will separate from the soln. Filter, and wash the precipitate twice with 10 cc of alcohol, 90% by volume, and then with alcohol, 70% by volume, taking care to maintain the arachidic acid and the wash solns at a definite temp. in order to apply the solubility corrections given below. Dissolve the arachidic acid upon the filter with boiling absolute alcohol, evaporate to dryness in a weighed dish, dry, and weigh. Add to the weight 0.0025 g for each 10 cc of 90% alcohol used in the crystallization and washing, if conducted at 15°; if conducted at 20°, add 0.0045 g for each 10 cc. The melting point of arachidic acid thus obtained is 71–72. Twenty times the weight of arachidic acid will give the approximate quantity of peanut oil present. Arachidic acid has a characteristic appearance and may be identified under the microscope. As little as 5–10% of peanut oil can be detected by this method.

## 41

COLD TEST<sup>22</sup>—TENTATIVE

(Applicable to all salad oils except olive oil.)

Fill a 4 oz sample bottle with the oil at a temp. of 25°, insert the cork stopper tightly, and seal with paraffin. Submerge the bottle completely in a bucket containing finely cracked ice and add H<sub>2</sub>O until it rises to the top of the bottle. Keep the bucket filled solidly with the cracked ice by removing any excess H<sub>2</sub>O and adding ice when necessary. At the end of 5 hours remove the bottle and examine the oil. If it is properly wintered, the sample will be brilliant, clear, and limpid.

## SESAME OIL

## 42

*Baudouin Test—Official*

Dissolve 0.1 g of finely powdered sugar in 10 cc of HCl, add 10 cc of the oil to be tested, shake thoroly for 1 min., and allow to stand for 10 min. In the presence of even a very small admixture of sesame oil, the aqueous soln is colored crimson. It should be observed that some olive oils, especially those of African or Spanish origin, give pink or crimson colors. These can be readily differentiated from the color due to sesame oil by the modified Villavecchia test, 43.

## 43

*Modified Villavecchia Test<sup>24</sup>—Official*

Add 2 cc of furfural to 100 cc of 95% alcohol. Mix thoroly 0.1 cc of this soln with 10 cc of HCl and 10 cc of the oil to be tested by shaking them together in a test tube for 15 seconds. Allow the mixture to stand for 10 min., observe color, add 10 cc of H<sub>2</sub>O, shake, and again observe the color. If the crimson color disappears, sesame oil is not present. (As furfural gives a violet tint with HCl, it is necessary to use the very dilute soln specified.)

44 DETECTION OF FOREIGN FATS CONTAINING TRISTEARIN IN LARD<sup>25</sup>—TENTATIVE

Weigh 5 g of the melted and filtered lard into a glass-stoppered cylinder and add 20 cc of warm acetone. Mix well, taking care that the soln is clear and has a temp. above 30°. Let stand at a constant temp. of 30° for 16–18 hours. A fine mass of crystals occupying a volume of not more than 3 cc should then be found at the bottom of the cylinder. Should the volume of crystals materially exceed 3 cc, take a smaller quantity of lard (3–4 g) for a new test. Should no crystals be deposited, as may be the case with soft or oily lard, absence of tristearin is indicated. Decant the supernatant acetone soln from the crystallized glycerides. Add warm (30–35°) acetone in three portions of 5 cc each from a small wash bottle, taking care not to break up the deposit in washing, and decant the first two portions. Actively agitate the third portion in the cylinder, and by a quick movement transfer the crystals to a small filter paper. Using the wash bottle, wash the crystals with 5 successive small portions of the warm acetone and remove excess acetone by suction. Spread out the paper and its contents, breaking up any large lumps and allow to dry in air at room temp. Thoroly comminute the mass and take the melting point of the crystals in a closed 1 mm tube, using an apparatus similar to that indicated under 15. Heat the H<sub>2</sub>O in the beaker rapidly to about 55° and maintain this temp until the thermometer carrying the melting point tube registers 50°, then heat again and raise the temp. of the outer bath rather quickly to 67°. Remove the burner. The melting point is reached when the fused substance becomes perfectly clear and transparent. When the melting point of the glycerides obtained by this method is below 63.6° the presence of beef fat or other fat containing tristearin should be suspected, and a melting

point of 63.2° or lower is evidence that the sample is not pure lard. It is advisable to carry out the method with a control sample of pure lard.

The conclusion indicated by the melting point may be confirmed by taking the melting point of the fatty acids prepared from the glycerides. After determining the melting point, transfer the crystallized glycerides to a 50 cc beaker, add 25 cc of approximately 0.5 *N* alcoholic KOH, and heat on a steam bath until saponification is complete. Pour the soln into a separatory funnel containing 200 cc of H<sub>2</sub>O, acidify, add 75 cc of ether, shake, and let stand. Draw off the aqueous acid layer and wash the ether soln at least 3 times with H<sub>2</sub>O. Transfer the ether soln to a clean dry 50 cc beaker, volatilize ether on the steam bath, and finally dry the acids at 100°. After about 2 hours, determine the melting point.

Conclusions may be confirmed further by precise determinations of the mean molecular weight of the separated fatty acids. Use a 0.5–0.2 *N* standard KOH soln and dissolve them in colorless, redistilled alcohol, which has been carefully neutralized immediately before use. If the sample is pure lard, the mean molecular weight of the fatty acids should correspond closely to that of the fatty acids of  $\alpha$ -palmitodistearin, 274.67. If the sample is impure, the mean molecular weight should tend to approach that of the fatty acids from tristearin, 284.

#### FISH OIL AND MARINE ANIMAL OILS IN THE PRESENCE OF VEGETABLE OILS AND IN THE ABSENCE OF METALLIC SALTS

45

##### *Qualitative Test—Tentative*

Dissolve in a test tube about 6 g of the oil in 12 cc of a mixture of equal parts of CHCl<sub>3</sub> and glacial acetic acid. Add Br, dropwise, until a slight excess is indicated by the color, keeping the soln at about 20°. Allow the mixture to stand 15 min. or more and then place the test tube in boiling H<sub>2</sub>O. If vegetable oils only are present, the soln will be perfectly clear, but fish oils will remain cloudy owing to the presence of insoluble bromides.

46

#### COLORING MATTERS—TENTATIVE

Into each of four 500 cc separators measure out four 100 cc portions of the oil and dilute each funnel with 100 cc of low-boiling gasoline. Extract two or three times with 50 cc portions of 2 *N* Na<sub>2</sub>CO<sub>3</sub>, passing same successively thru each funnel. If a yellow or pink color is obtained, test for Sudan G, annatto, or turmeric. Then extract the oil soln successively with three 50 cc portions of a mixture consisting of HCl and glacial acetic acid (1+5). A pink or red lower layer indicates aniline yellow (7) [15], butter yellow (16) [19], yellow AB (—) [21], or yellow OB (—) [61]. For detailed separation see **XXI**.

#### COTTONSEED<sup>26</sup>

47

##### SAMPLING

Use a portion of the sample of cottonseed received at the laboratory of approximately 1000 g (2½ lbs.) of cleaned seed (to be known as the laboratory sample). It should be received sealed in an air-tight container and should be accompanied by a statement, certified by the sampler, giving the weight of the original sample and of the foreign matter separated by him.

48

##### FOREIGN MATTER

Examine the laboratory sample immediately and if found not to have been thoroly cleaned carefully weigh and reclean by use of a 6-mesh screen and by the hand-picking of all remaining particles of foreign matter. Calculate the percentage

of foreign matter by dividing the weight of the foreign matter reported by the sampler by the weight of the original sample and correcting the result by adding the percentage of foreign matter found in the laboratory sample.

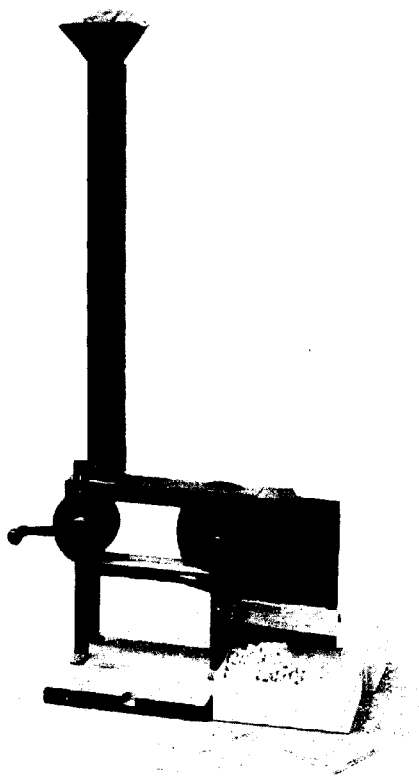


FIG. 39.—MELOY COTTONSEED MIXER AND DIVIDER

49

MIXING AND QUARTERING

Mix the cleaned laboratory sample and quarter by one of the following methods:

(a) Place the sample in an approved mechanical mixer (MacLellan mixer No. 00-S) and mix by revolving 10 times at the rate of 5 revolutions a minute. After mixing, empty the sample onto a large piece of paper, press, and quarter with a large spatula. Separate quadrants Nos. 1 and 3 from quadrants Nos. 2 and 4. Im-

mediately return quadrants Nos. 1 and 3 to the original container, seal, and retain as a referee sample. Preserve quadrants Nos. 2 and 4 in an air-tight container for analysis. When finally using them for analysis, again place on a piece of paper and requarter until the combining of opposite quadrants yields two samples of approximately 120 g each. Use one of these samples for the determination of free fatty acids. Further quarter the other sample to yield two samples of approximately 60 g each, using one of these for the moisture determination and the other for oil and ammonia determinations.

(b) Mix and quarter the laboratory sample by passing the entire sample thru the cottonseed sample divider as illustrated (Fig. 39). (A perfect mix can be accomplished by passing the entire sample thru the divider 2 or 3 times.) After mixing, reserve one of the two portions resulting from the division, of approximately 500 g each, as a referee sample. Again pass the second portion thru the divider. Reserve one of the resulting two portions, of approximately 250 g each, for emergency check analysis. Return the second portion thru the divider and use one of the resulting portions, of approximately 125 g each, for the determination of free fatty acids. Again divide the remaining portion to yield two portions of approximately 62 g each and use one portion for the moisture determination and the other for the oil and ammonia determinations.

## 50

## MOISTURE

Use one of the following methods:

(a) Carefully crack each seed coat by means of an approved laboratory crimper. Weigh duplicate samples of about 5 g each into Al dishes 2 in. in diameter and  $\frac{3}{4}$  in. high, fitted with covers. Dry for 5 hours at 101° in a jacketed oven or a forced-draft circulatory oven of approved type. Do not open the oven during the drying period; if this is unavoidable extend the actual time sufficiently to offset any temporary cooling due to opening the oven. When the drying period is over, place the cover on the dish and place the dish in an efficient desiccator until cool (30 min.). Weigh the sample and calculate the loss in weight as moisture.

(NOTE.—For use in cracking the seed coats an ordinary tinner's crimper, when properly adjusted, is approved. A more satisfactory cracker, which may be used also for hulling dried cottonseed for the separation of the kernels or meats, can be made by substituting steel rollers for the rubber-covered rollers of a clothes wringer, the lower roller being knurled and the upper roller grooved.)

(b) Weigh into shallow moisture dishes duplicate samples of about 5 g each of the whole uncracked seed and distribute the seed evenly. Place the uncovered dishes containing the samples in the oven specified under (a) at 101° for 12–16 hours, or most conveniently, overnight. Remove the dishes from the oven, cover, cool in an efficient desiccator 30 min., and weigh. Calculate the loss in weight as moisture.

## 51

## PREPARATION OF SEED FOR OIL AND AMMONIA DETERMINATIONS

Dry the approximately 60 g portion, quartered out for the purpose, for 2 hours at 130°,  $\pm 3^\circ$ , in an approved type of uniform forced-draft circulatory oven. Absorb into the inner walls and bottom of a porous earthenware vessel (such as a 3 in. flower pot) 1.5 cc of HCl. (The acid should be well distributed over the sides and bottom of the pot. When the acid has been absorbed the pot should appear dry; if it does not it was probably not in proper condition for this use.)

Place the dried seed in the pot, cover with a watch-glass, and place it in a fuming oven (a well-ventilated noncorrosive oven capable of reaching and maintaining a

temp. of  $125^{\circ} \pm 5^{\circ}$ ) for 1 hour. (When the seed is fumed, the lint should be loose and brittle, but not scorched.) Grind the sample in a Bauer mill (No. 148 laboratory mill with No. 6912 plate), which has been adjusted to produce a fine meal. After grinding, open the mill and carefully brush out all remaining ground seed onto a large sheet of smooth paper. (There should be practically no loss of material in grinding.)

Mix the ground sample thoroly, preferably by placing it in a 2 quart Mason fruit jar together with a large rubber stopper. Replace the cover and shake violently until the ground material is thoroly mixed; then transfer to a well-stoppered bottle or container of just sufficient size to hold the material tightly so as to prevent percolation or vertical segregation of the components.

52

## MOISTURE IN GROUND SAMPLE

Weigh 5 g of the fumed and ground sample into a moisture dish and dry at  $101^{\circ}$  for 2 hours in the oven specified in 50(a). Calculate loss in weight as moisture content.

## OIL

53

## APPARATUS

(a) *Extractor*.—Butt type.

(b) *Condensers*.—Allihn's with 12 in. jackets, fitted with cork connections.

54

## REAGENT

*Petroleum ether*.—Initial boiling temp.,  $35-40^{\circ}$ ; dry-flask end point,  $50-60^{\circ}$ ; at least 95% distilling under  $55^{\circ}$ , and not over 85% distilling under  $40^{\circ}$ ; sp. gr. at  $60^{\circ}\text{F}$ ., 0.630-0.675; color, water white; evaporation residue, not over 0.002% by weight; doctor test, sweet; copper-strip corrosion test, noncorrosive; trace only of unsaturated compounds permitted.

Make the distillation test according to the method of the American Society for Testing Materials (standard method D86-27 for distillation test of gasoline) and make a blank by evaporating 250 cc with about 0.25 g of stearin or other hard fat (previously brought to constant weight by heating) and drying as in the actual determination. The blank must not exceed a few mg.

55

## DETERMINATION

Weigh accurately duplicate samples of 4-5 g of the fumed and ground seed and spread each portion in a thin layer on a 150 mm filter paper (Reeve-Angel No. 211 or equivalent grade); fold the paper over the sample at a point about one-quarter the distance from each of two opposite sides to the center; wrap by coiling from one of the unfolded sides into a cylinder; and rewrap in a second paper or papers in such manner as to prevent escape of the meal, leaving the top of the second paper open like a thimble. Place a piece of absorbent cotton in the top of the thimble to distribute the dropping ether. Place 25 cc of the petroleum ether in a tared flask, 125 cc capacity, and extract the sample for 4 hours. (The ether should drop on the center of the thimble at a rate of at least 150 drops per min., and the volume of the solvent should be kept approximately constant.) Then evaporate the solvent until no trace remains, cool the sample to room temp., and weigh. As the last traces of ether are sometimes difficult to detect by odor, in case of doubt evaporate for an hour, or longer, until constant weight is obtained. Calculate the oil content as shown in the following example:

Petroleum ether extract.....	1.025 grams
Original moisture 12.2% plus total foreign matter up to and including 1.0%.....	12.2+0.8=13.0
Second moisture.....	2.6

$$\frac{1.025}{5} \times \frac{87}{97.4} = 18.3\% \text{ of oil.}$$

56

## AMMONIA

Digest 1.7034 g of the sample in a 650–800 cc Kjeldahl flask with approximately 0.5 g of metallic Hg or 0.7 g of HgO, 10 g of Na<sub>2</sub>SO<sub>4</sub> or K<sub>2</sub>SO<sub>4</sub>, and 25 cc of H<sub>2</sub>SO<sub>4</sub> (sp. gr. 1.84). Place the flask in an inclined position and heat below the boiling point of the acid from 5 to 15 min., or until frothing has ceased. Increase the temp. and continue digestion until the liquid becomes colorless, or until complete digestion is obtained. From this point use the regular Kjeldahl method, omitting the addition of KMnO<sub>4</sub>.

After cooling the mixture add about 300 cc of distilled H<sub>2</sub>O, a few granules of Zn to keep the contents of the flask from bumping, and 25 cc of the K<sub>2</sub>S or Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> soln, or a sufficient quantity to precipitate all the Hg. After mixing thoroly, add 60 cc of caustic soda soln (sp. gr. 1.50), or sufficient to make strongly alkaline, pouring the soln down the side of the flask so that it does not mix at once with the acid soln. Connect the flask with a condenser of block tin, mix the contents of the flask by shaking, and distil into an accurately measured quantity of standard H<sub>2</sub>SO<sub>4</sub> soln (0.5 N) to which has been added 50 cc of H<sub>2</sub>O, until at least 200 cc of distillate is obtained, taking care that the delivery tube reaches below the level of the standard acid. Add about 1 cc of a 0.2% aqueous soln of sodium alizarin sulfonate as the indicator. (Either cochineal or methyl red may be used as the indicator, but with methyl red the soln is titrated hot.) Then titrate the distillate with a standard 0.25 N NaOH soln.

By using 1.7034 g of sample for the analysis, the number of cc of 0.5 N acid required for the neutralization of the distilled NH<sub>3</sub>, divided by 2, gives the percentage of NH<sub>3</sub>.

Make a blank test on all reagents and correct the titration of the above distillate accordingly.

57

## CALCULATION

Example:

	cc
0.5 N HCl measured into flask.....	10.00
0.5 N HCl for blank test on reagents.....	0.06
0.25 N NaOH used in titration.....	2.68

$$\frac{10 - 0.06 - 2.68}{2} = 4.30\% \text{ ammonia in fumed seed}$$

Original moisture.....	8.1
Moisture in fumed seed.....	2.0
Foreign matter, up to 1.0.....	0.9

$$\frac{4.30 \times 0.91}{0.98} = 3.99\% \text{ ammonia in original seed}$$

58

## FREE FATTY ACIDS

Heat 200 g of the original clean sample of seed for 30–40 min. at a temp. of 100–105°, and cool. Pass the cooled seed thru a Bauer mill set to merely crack all the



seed. Separate the meats from the hulls by the use of a 4-6 mesh screen. Grind the meats in a Ruswine No. 1 food chopper equipped with 16-tooth blade. Thoroughly mix the sample and pass thru a 15-mesh screen so as to remove any remaining lint or hulls. (Proper grinding and complete separation of meats from hulls are essential points in obtaining concordant results.) Without undue loss of time quarter the thoroughly mixed ground meats so as to obtain at least a 40 g sample. Extract this sample by cold percolation in the following manner: Place the lower disk from a Knorr extraction apparatus in a Butt tube and place on it a layer of asbestos fiber suspended in petroleum ether. (A satisfactory mat should allow none of the meats to pass thru but should allow the extracting solvent to flow thru at about 150 drops per min.) Place the sample in the prepared tube, and add 50 cc of petroleum ether followed by two portions of 25 cc each of petroleum ether, allowing each portion to flow thru before adding the next portion. Allow the extracted oil to remain on the steam bath for 1½ hours to completely remove all trace of the solvent. Weigh 7.05 g of the oil into a titrating flask; add 30 cc of neutralized alcohol, 10 cc of petroleum ether, 1 cc of 1% phenolphthalein; and titrate the free fatty acid of the oil with standard 0.25 N alkali. Shake the flask vigorously during the titration, and take as the end point a permanent pink which persists for at least one min.

$$\% \text{ F.F.A.} = \frac{28.2 \times \text{normality of alkali} \times \text{cc used}}{\text{weight of oil}}$$

If results indicate a free fatty acid content of 4% or higher, duplicate the complete test.

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## XXXII. PRESERVATIVES AND ARTIFICIAL SWEETENERS

### SALICYLIC ACID

1

#### PREPARATION OF SAMPLE—OFFICIAL

(a) *Non-alcoholic liquids*.—Many liquids may be extracted directly as described under 2 or 4 without further treatment. If troublesome emulsions form during extraction, pipet 100 cc into a 250 cc volumetric flask, and add about 5 g of NaCl, shaking until dissolved. Make up to the mark with 95% alcohol, shake vigorously, allow the mixture to stand for 10 min. with occasional shaking, filter, and treat an aliquot of the filtrate as directed under (b).

(b) *Alcoholic liquids*.—Make 200 cc of the sample alkaline with approximately 10% NaOH soln, using litmus paper as an indicator, and evaporate on a steam bath to about  $\frac{1}{2}$  its original volume. Dilute to the original volume with H<sub>2</sub>O and filter if necessary.

(c) *Solid or semi-solid substances*.—Grind the sample and mix thoroly. Transfer a convenient quantity (50–200 g according to the consistency of the sample) to a 500 cc volumetric flask, add sufficient H<sub>2</sub>O to make a volume of about 400 cc, and shake until the mixture becomes uniform. Add 2–5 g of CaCl<sub>2</sub> and shake until dissolved; render distinctly alkaline with approximately 10% NaOH soln, using litmus paper as an indicator; fill to the mark with H<sub>2</sub>O, shake thoroly, allow to stand for at least 2 hours, shaking frequently, and filter.

#### QUALITATIVE TESTS

2

#### Ferric Chloride Test—Official

Introduce 50 cc of the sample or an equivalent quantity of an aqueous extract, prepared as directed under 1, into a separatory funnel; add 1/10 its volume of HCl (1+3) and extract with 50 cc of ether. If the mixture emulsifies, add 10–15 cc of petroleum ether (b.p. below 60°) and shake. If this treatment fails to break the emulsion, whirl the mixture in a centrifuge, or allow it to stand until a considerable portion of the aqueous layer has separated; run off the latter, shake vigorously, and again allow to separate. Wash the ether layer with two 5 cc portions of H<sub>2</sub>O, evaporate the greater portion of the ether in a porcelain dish on a steam bath, allow the remainder to evaporate spontaneously, and add a drop of 0.5% neutral FeCl<sub>3</sub> soln. A violet color indicates salicylic acid.

If coloring matter or other interfering substance is present in the residue left after evaporation of the ether, purify the salicylic acid by one of the following methods:

(a) Dissolve the original residue from the ether extract, obtained as directed above, in about 25 cc of ether; transfer this soln to a separatory funnel and shake with an equal volume of H<sub>2</sub>O made distinctly alkaline with several drops of 10% NH<sub>4</sub>OH. Allow to separate, filter the aqueous layer thru a wet filter into a porcelain dish, evaporate almost to dryness, and test the residue as directed previously.

(b) Dry the original residue from the ether extract, obtained as directed above, in a desiccator over H<sub>2</sub>SO<sub>4</sub> and extract with several 10 cc portions of CS<sub>2</sub> or petroleum ether (b.p. below 60°), rubbing the contents of the dish with a glass rod and filtering the successive portions of the solvent thru a dry paper into a second porcelain dish. Evaporate the greater portion of the solvent on a steam bath, allow the remainder to evaporate spontaneously, and test the residue as directed previously.

(c) By means of a few cc of ether, transfer the original residue from the ether extract, obtained as directed above, to a small porcelain crucible, and allow the solvent to evaporate spontaneously. Cut a hole sufficiently large in an asbestos board to admit about  $\frac{1}{4}$  of the crucible, cover with a small, round-bottomed flask filled with cold  $H_2O$ , and heat over a small Bunsen flame until any salicylic acid present has sublimed and condensed upon the bottom of the flask. Test the sublimate as directed previously.

## 3

*Jorissen Test*<sup>1</sup>—Official

Dissolve the residue from the ether extract, obtained as directed under 2, or, if impurities are present, the purified material obtained as directed under 2(a), (b), or (c), in a little hot  $H_2O$ . Cool 10 cc of the soln in a test tube; add 4 or 5 drops of 10%  $KNO_3$  soln, 4 or 5 drops of acetic acid (approximately 50% soln), and 1 drop of 1%  $CuSO_4$  soln; mix thoroly, boil the liquid for half a min., and allow to stand for 2 min. In the presence of salicylic acid a Bordeaux-red color develops.

## QUANTITATIVE METHOD—OFFICIAL

## 4

## EXTRACTION

Transfer to a separatory funnel 100 cc of the sample, or that quantity of a soln prepared as directed under 1 which represents not less than 20 g of the original material. Neutralize to litmus with  $HCl$  (1+3) and add an excess of  $HCl$  equivalent to 2 cc of acid for each 100 cc of soln. Extract with 4 separate portions of ether, using for each extraction a volume of ether equivalent to  $\frac{1}{2}$  the volume of the aqueous layer. If an emulsion forms on shaking, this may usually be broken by adding a little ( $\frac{1}{2}$  the volume of the ether layer) petroleum ether (b.p. below  $60^\circ$ ) and shaking again, or by centrifuging. If a small quantity of emulsion still persists, allow it to remain with the aqueous layer, where frequently it is broken during the next extraction. If an emulsion remains after the fourth extraction, separate it from the clear ether and the clear aqueous layer and extract it separately with 2 or 3 small portions of ether. Combine the ether extracts, wash with a volume of  $H_2O$  equal to  $1/10$  of the total volume of ether extracts, allow the layers to separate, and reject the aqueous layer. Wash in this way until the aqueous layer after separation yields a yellow color upon the addition of methyl orange soln and 2 drops of 0.1  $N$   $NaOH$ . Distil slowly the greater part of the ether, transfer the remainder to a porcelain dish, and allow it to evaporate spontaneously. If no interfering substances are present, proceed as directed under 5; if interfering substances are present, purify the residue by one of the following methods:

(a) Thoroly dry the residue in vacuo over  $H_2SO_4$ . Extract it 10 times with 10-15 cc portions each of  $CS_2$  or petroleum ether (b.p. below  $60^\circ$ ), rubbing the contents of the dish with a glass rod, and filter the successive portions of the solvent thru a dry filter into a porcelain dish. Test the extracted residue with a drop of 2% ferric alum soln, and if it gives a reaction for salicylic acid, dissolve it in  $H_2O$ ; acidify the soln with  $HCl$  (1+3), extract with ether, evaporate, extract the dry residue thus obtained with  $CS_2$  or petroleum ether, and add to the extract first obtained. Distil the greater portion of the  $CS_2$  or petroleum ether and allow the remainder to evaporate spontaneously. Proceed as directed under 5.

(b) Dissolve the residue in 40-50 cc of ether. Transfer the ether soln to a separatory funnel and extract with 3 successive 15 cc portions of 1%  $NH_4OH$ . (If fat is known to be present in the original ether extract, extract the latter directly with 4 portions of the  $NH_4OH$  instead of 3.) Combine the alkaline aqueous extracts, acid-

ify, again extract with ether, and wash the combined ether extracts as directed previously. Slowly distil the greater portion of the ether, allow the remainder to evaporate spontaneously, and proceed as directed under 5.

## 5

## DETERMINATION

Dissolve the residue, 4, in a small quantity of hot  $\text{H}_2\text{O}$ , and after cooling dilute to a definite volume (usually 50 or 100 cc). If the soln is not clear, filter thru a dry filter. Dilute aliquots of the soln and treat with 0.5%  $\text{FeCl}_3$  soln or 2% ferric alum soln until the maximum color is developed. Generally a few drops will suffice.

(The ferric alum soln should be boiled until a precipitate appears, allowed to settle, and filtered. The acidity of the soln is slightly increased in this manner, but the soln remains clear for a considerable time, and the turbidity caused by its dilution with  $\text{H}_2\text{O}$  is much less and does not appear so soon as when the unboiled soln is used. This turbidity interferes with the exact matching of the color.)

Compare the colors developed with the color obtained when a standard salicylic acid soln (containing 1 mg of salicylic acid in 50 cc) is similarly treated, using Nessler tubes or a colorimeter. In either case, and especially with  $\text{FeCl}_3$ , avoid an excess of the reagent, altho an excess of 0.5 cc of 2% ferric alum soln may be added to 50 cc of the comparison soln of salicylic acid without vitiating the results.

## BENZOIC ACID

## QUALITATIVE TESTS

## 6

## I. Preliminary Test—Official

Extract benzoic acid as directed under 2 or 4. If benzoic acid is present in considerable quantity, it will crystallize from the ether in shining leaflets having a characteristic odor on warming. Dissolve the crystalline deposit in hot  $\text{H}_2\text{O}$ , divide into 2 portions, and test as directed in 7 or 8. The deposit may also be purified as directed under 2(c) and the melting point determined.

## 7

## II. Ferric Chloride Test—Official

Make the soln from 6 alkaline with a few drops of  $\text{NH}_4\text{OH}$ , expel the excess of  $\text{NH}_3$  by evaporation, dissolve the residue in a few cc of hot  $\text{H}_2\text{O}$ , filter if necessary, and add a few drops of a neutral 0.5%  $\text{FeCl}_3$  soln. A salmon-colored precipitate of ferric benzoate indicates the presence of benzoic acid.

## 8

## III. Modified Mohler Test—Official

(The presence of phenolphthalein interferes with this test.)

Add to the  $\text{H}_2\text{O}$  soln, prepared as described under 6, 1 or 2 drops of about 10%  $\text{NaOH}$  soln and evaporate to dryness. To the residue add 5–10 drops of  $\text{H}_2\text{SO}_4$  and a small crystal of  $\text{KNO}_3$ . Heat for 10 min. in a bath (glycerol) at  $120$ – $130^\circ$  (the temp. must not exceed  $130^\circ$ ). After cooling, add 1 cc of  $\text{H}_2\text{O}$  and make distinctly ammoniacal. Boil the soln to decompose any  $\text{NH}_4\text{NO}_3$  that might have been formed. Cool, and add a drop of fresh, colorless  $(\text{NH}_4)_2\text{S}$  soln, but do not allow the layers to mix. A red-brown ring indicates benzoic acid. On mixing the color diffuses thru the whole liquid, and on heating finally changes to greenish yellow. This change differentiates benzoic acid from salicylic acid or cinnamic acid. The salicylic and cinnamic acids form colored compounds that are not destroyed by heating.

## QUANTITATIVE METHODS—OFFICIAL

## PREPARATION OF SAMPLE

9

*General Method*

Mix the sample thoroly, grinding if solid or semi-solid. Transfer 150 cc or 150 g to a 500 cc volumetric flask, add enough pulverized NaCl to saturate the H<sub>2</sub>O in the sample, make alkaline to litmus paper with 10% NaOH soln or with milk of lime, and dilute to the mark with saturated NaCl soln. Shake thoroly, allow to stand for at least 2 hours, with frequent shaking, and filter.

If the sample contains large quantities of fat, portions of which may contaminate the filtrate, add a few cc of the NaOH soln to the filtrate and extract with ether before proceeding as directed under 11. If alcohol is present, proceed as directed under 10(c). If the sample contains large quantities of matter precipitable by salt soln, proceed as directed under 10(d).

10

*Special Methods*

(a) *Ketchup*.—To 150 g of the ketchup add 15 g of pulverized NaCl, and transfer the mixture to a 500 cc volumetric flask, rinsing with about 150 cc of saturated NaCl soln. Make slightly alkaline to litmus paper with 10% NaOH soln and fill to the mark with saturated NaCl soln. Allow to stand for at least 2 hours, shaking frequently. Squeeze thru a heavy muslin bag and filter.

(b) *Jellies, jams, preserves, and marmalades*.—Digest 150 g of the sample in about 300 cc of saturated NaCl soln. Add 15 g of pulverized NaCl. Make alkaline to litmus paper with milk of lime. Transfer to a 500 cc volumetric flask and dilute to the mark with saturated NaCl soln. Allow to stand for at least 2 hours, shaking frequently; centrifuge if necessary, and filter.

(c) *Cider containing alcohol, and similar products*.—Make 250 cc of the sample alkaline to litmus paper with 10% NaOH soln and evaporate on a steam bath to about 100 cc. Transfer the sample to a 250 cc volumetric flask, add 30 g of pulverized NaCl, and shake until dissolved. Dilute to the original volume, 250 cc, with saturated NaCl soln; allow to stand for at least 2 hours, shaking frequently, and filter.

(d) *Salted or dried fish*.—Wash 50 g of the ground sample into a 500 cc volumetric flask with H<sub>2</sub>O. Make slightly alkaline to litmus paper with 10% NaOH soln and dilute to the mark with H<sub>2</sub>O. Allow to stand for at least 2 hours, shaking frequently, and filter. Pipet as large a measured portion of the filtrate as possible (at least 300 cc) into a second 500 cc flask, adding 30 g of the pulverized NaCl for each 100 cc of soln. Shake until the NaCl has dissolved and dilute to the mark with saturated NaCl soln. Mix thoroly and filter off the precipitated protein and other extraneous matter.

11

## DETERMINATION

Pipet a convenient portion (100–200 cc) of the filtrate obtained under 9 or 10 into a separatory funnel. Neutralize the soln to litmus paper with HCl (1 + 3) and add an excess of 5 cc of the same acid. In the case of salted fish a precipitation of protein matter usually occurs on acidifying, but the precipitate does not interfere with the extraction. Extract carefully with CHCl<sub>3</sub>, using successively portions of 70, 50, 40, and 30 cc. To avoid the formation of an emulsion, shake cautiously each time, using a rotary motion. The CHCl<sub>3</sub> layer usually separates readily after standing a few min. If an emulsion forms, break it by stirring the CHCl<sub>3</sub> layer with a glass rod, by drawing it off into a second funnel and giving one or two sharp shakes from one end of the funnel to the other, or by centrifuging for a few min. As this is a pro-

gressive extraction, draw off carefully as much of the clear  $\text{CHCl}_3$  soln as possible after each extraction, but do not draw off any of the emulsion with the  $\text{CHCl}_3$  layer. If this precaution is taken, the  $\text{CHCl}_3$  extract need not be washed.

Transfer the combined  $\text{CHCl}_3$  extracts to a porcelain evaporating dish, rinse the container several times with a few cc of  $\text{CHCl}_3$ , and evaporate to dryness at room temp. in a current of dry air.

The extract may also be transferred from the separatory funnel to a 300 cc Erlenmeyer flask, the separatory funnel being rinsed 3 times with 5-10 cc portions of  $\text{CHCl}_3$ . Distil very slowly at a low temp. to about  $\frac{1}{2}$  the original volume. Then transfer the residue to a porcelain evaporating dish, rinsing the flask 3 times with 5-10 cc portions of  $\text{CHCl}_3$ , and evaporate to dryness at room temp. in a current of dry air.

Dry the residue overnight (or until no odor of acetic acid can be detected if the product is a ketchup) in a desiccator containing  $\text{H}_2\text{SO}_4$ . Dissolve the residue of benzoic acid in 30-50 cc of 95% alcohol neutral to phenolphthalein; add about  $\frac{1}{2}$  this volume of  $\text{H}_2\text{O}$  and 1 or 2 drops of phenolphthalein indicator, II, 10(d); and titrate with 0.05 N NaOH. 1 cc of 0.05 N NaOH = 0.0072 g of anhydrous Na benzoate.

### SACCHARIN

#### 12

##### PREPARATION OF SAMPLE—OFFICIAL

(a) *Fruit juices and sirups.*—Transfer 100-200 g of the sample to a 250 cc volumetric flask by means of a little  $\text{H}_2\text{O}$  and dilute to about 200 cc with  $\text{H}_2\text{O}$ . Add 5 cc of glacial acetic acid and mix. Add a slight excess of 20% neutral Pb acetate soln, mix thoroly, dilute to the mark with  $\text{H}_2\text{O}$ , again mix thoroly, and filter.

(b) *Alcoholic liquids.*—Heat 100-200 cc of the liquid on a steam bath to remove alcohol, this being accomplished in most cases by evaporating to  $\frac{1}{2}$  the original volume. With heavy sirups, dilute the liquid with an equal volume of  $\text{H}_2\text{O}$  before beginning the evaporation. After the alcohol has been removed, transfer to a 250 cc volumetric flask and proceed from this point as directed under (a).

(c) *Solid or semi-solid preparations.*—Transfer 50-75 g of the sample to a 250 cc volumetric flask by means of a little hot  $\text{H}_2\text{O}$  and add sufficient boiling  $\text{H}_2\text{O}$  to make the volume about 200 cc. Allow the mixture to stand for 2 hours, shaking occasionally. Then add 5 cc of glacial acetic acid, mix thoroly, add a slight excess of 20% neutral Pb acetate soln, dilute to the mark with cold  $\text{H}_2\text{O}$ , mix, allow to stand for 20 min., and filter.

#### 13

##### QUALITATIVE TEST—OFFICIAL

(a) Acidify 50 cc of non-alcoholic liquid foods or the aqueous extract of 50 g of a solid or semi-solid product, prepared as directed under 12, with HCl and extract 3 times with 25 cc portions of ether. Wash the combined ether extracts once with 5 cc of  $\text{H}_2\text{O}$ , transfer to a small beaker or evaporating dish, allow the ether to evaporate spontaneously, and taste the residue. The presence of as little as 20 mg of saccharin per liter or kg of the original sample can usually be detected by its sweet taste. Confirm by heating with NaOH and detecting the salicylic acid formed thereby as directed under (b).

(b) Acidify 50 cc of a non-alcoholic liquid food, or an equivalent quantity of an aqueous extract prepared as directed under 12, with HCl and extract with 3 portions of ether as directed under (a). Dissolve the residue remaining after evaporation of the ether in a little hot  $\text{H}_2\text{O}$  and test a small portion of the soln for salicylic acid as directed under 2 or 3. Dilute the remainder of the soln to about 10 cc and add 2 cc of  $\text{H}_2\text{SO}_4$  (1+3). Heat to boiling and add a slight excess of 5%  $\text{KMnO}_4$  soln drop-

wise; partly cool the soln, dissolve approximately 1 g of NaOH in it, and filter the mixture into an Ag dish (silver crucible lids are well adapted to the purpose). Evaporate to dryness and heat for 20 min. at 210–215°. Dissolve the residue in  $H_2O$ , acidify with HCl, and test the ether extract for salicylic acid as directed under 2 or 3. By this method all the so-called "false saccharin" and any salicylic acid naturally present (also added salicylic acid when not present in too large a quantity) are destroyed, whereas 5 mg of saccharin per liter is detected with certainty.

#### QUANTITATIVE METHODS

##### 14

##### *I. General Method—Official*

Transfer 150 cc of the filtrate obtained under 12 to a separatory funnel, add 15 cc of HCl, and extract 3 times with 80 cc portions of ether, shaking the separatory funnel for 2 min. each time. Wash the combined ether extracts once with 5 cc of  $H_2O$ , remove the ether by distillation, and transfer the residue to a Pt crucible by means of a little ether; or, if substances difficultly soluble in ether are present, use alternately small portions of  $H_2O$  and ether. Evaporate the ether on a steam bath, add to the residue 2–3 cc (or enough to make the mixture strongly alkaline) of a 10%  $Na_2CO_3$  soln, rotate so that all the saccharin is brought in contact with the soln, and evaporate to dryness on a steam bath. To the dry residue in the crucible add 4 g of a mixture of equal parts of anhydrous  $Na_2CO_3$  and  $K_2CO_3$ . Heat gently at first and then to complete fusion for 30 min. over an alcohol or other S-free flame. The fusion may be conducted by closely fitting the crucible into a hole cut into a piece of heavy asbestos board so that  $\frac{1}{2}$  of the crucible projects above the asbestos, and heating the lower portion of the crucible by means of a large Bunsen, Meker, or similar burner. Cool, dissolve the melt in  $H_2O$ , add about 5 cc of Br water, acidify with HCl, filter, wash the paper with a little  $H_2O$ , dilute the filtrate and washings to about 200 cc, heat to boiling, and slowly add an excess of  $BaCl_2$  soln (approximately 10%). Allow to stand overnight, collect the  $BaSO_4$  on a filter or on a Pt Gooch crucible, wash until free from chlorides, dry, ignite, cool, and weigh. Correct the result thus obtained for any S present in the fusion mixture as found by a blank determination. Calculate the equivalent quantity of saccharin by multiplying the corrected weight of  $BaSO_4$  by 0.7844.

(Instead of the mixed Na and K carbonates, 3–4 g of  $Na_2O_2$  may be used for the fusion. In this case a Ni crucible must be used, and the time of fusion may be reduced to 5 min. The separation of a little  $PbCl_2$  during the extractions does not interfere with the accuracy of the method.)

##### 15

##### *II. Special Method—Official, First Action*

*In non-alcoholic beverages.*—Add 2 cc of HCl to 50 cc of the drink contained in a separatory funnel. Extract with two successive 50 cc portions of ether. Filter the ether extractions thru cotton, and wash the combined filtrates with approximately 5 cc of distilled  $H_2O$  to which has been added 1 drop of HCl.

Separate the ethereal layer and evaporate to dryness on a water bath. Add to the residue 5 cc of  $H_2O$  and 6 cc of HCl and evaporate the soln to about 1 cc on a hot plate with constant stirring. Again add 5 cc of distilled  $H_2O$  and 6 cc of HCl and evaporate to about 1 cc. Dilute to 50 cc with ammonia-free  $H_2O$  and dilute 5 cc of this soln to 25 cc with ammonia-free  $H_2O$ . Add 2.5 cc of Nessler's reagent, XXXVII, 10(a), and compare with  $NH_4Cl$  standards in the usual manner; 0.2923 g of dry  $NH_4Cl = 1$  g of saccharin, insoluble form,  $= 1.39$  g of the sodium salt of the Pharmacopoeia crystallizing with 2 molecules of  $H_2O$  of hydration. For convenience



prepare an  $\text{NH}_4\text{Cl}$  standard equivalent to 200 p.p.m. of the insoluble form of saccharin.

### BORIC ACID AND BORATES

16

#### QUALITATIVE TEST—OFFICIAL

*Preliminary test.*—Acidify the sample with  $\text{HCl}$  in the proportion of 7 cc of acid to each 100 cc of sample. In case of solid or pasty samples heat with enough  $\text{H}_2\text{O}$  to make sufficiently fluid before acidifying. Immerse a strip of turmeric paper in the acidified liquid, and allow the paper to dry spontaneously. If borax or  $\text{H}_3\text{BO}_3$  is present, the paper will acquire a characteristic red color, changed by  $\text{NH}_4\text{OH}$  to a dark blue-green, but restored by acid.

*Confirmatory test.*—Make about 25 g of the sample decidedly alkaline with lime  $\text{H}_2\text{O}$  and evaporate to dryness on a steam bath. Ignite the dry residue at a low red heat until the organic matter is thoroly charred. Cool, digest with about 15 cc of  $\text{H}_2\text{O}$ , and add  $\text{HCl}$  dropwise until the soln is distinctly acid. Immerse a piece of turmeric paper in the soln and allow it to dry without heat. In the presence of borax or  $\text{H}_3\text{BO}_3$ , the color change will be the same as described under the preliminary test.

17

#### QUANTITATIVE METHOD—OFFICIAL

Make 10–100 g of the sample (depending upon the nature of the material and the quantity of  $\text{H}_3\text{BO}_3$  present) distinctly alkaline with  $\text{NaOH}$  soln and evaporate to dryness in a Pt dish. Ignite the residue until the organic matter is thoroly charred, avoiding an intense red heat; cool, digest with about 20 cc of hot  $\text{H}_2\text{O}$ , and add  $\text{HCl}$  dropwise until the reaction is distinctly acid. Filter into a 100 cc volumetric flask and wash with a little hot  $\text{H}_2\text{O}$ . The volume of the filtrate should not exceed 50–60 cc. Return the filter containing any unoxidized C to the Pt dish, make alkaline by wetting thoroly with lime  $\text{H}_2\text{O}$ , dry on a steam bath, and ignite to a white ash. Dissolve the ash in a few cc of  $\text{HCl}$  (1+3) and add to the liquid in the 100 cc flask, rinsing the dish with a few cc of  $\text{H}_2\text{O}$ . To the combined solns, add 0.5 g of  $\text{CaCl}_2$  and a few drops of phenolphthalein indicator, then 10%  $\text{NaOH}$  soln until a permanent light pink color is produced. Finally dilute to the mark with lime  $\text{H}_2\text{O}$ , mix, and filter thru a dry filter. To 50 cc of the filtrate add 1 N  $\text{H}_2\text{SO}_4$ , II, 19(b), until the pink color disappears, then add methyl orange indicator, VI, 3(f), and continue the addition of the acid until the yellow color is changed to pink. Boil for about 1 min. to expel  $\text{CO}_2$ . Cool, and carefully add 0.2 N  $\text{NaOH}$  until the liquid assumes a yellow tinge, avoiding an excess of the alkali. (All the boric acid is now in a free state with no uncombined  $\text{H}_2\text{SO}_4$  present.) Add 1.2 g of neutral mannitol and a few drops of phenolphthalein indicator, note the buret reading, and again titrate the soln with the standard  $\text{NaOH}$  until a pink color develops. Add a little more mannitol, and if the pink color disappears continue the addition of the standard alkali until a pink color reappears. Repeat the alternate addition of mannitol and standard alkali until a permanent end point is reached. A volume of glycerol neutral to phenolphthalein equal to the volume of the soln to be titrated may be substituted for the mannitol. 1 cc of 0.2 N  $\text{NaOH}$  soln = 0.0124 g of boric acid.

### FORMALDEHYDE

18

#### PREPARATION OF SAMPLE—OFFICIAL

If the sample is solid or semi-solid, macerate 200–300 g of the material with about 100 cc of  $\text{H}_2\text{O}$  in a mortar. Transfer to a short-necked, 500–800 cc Cu or glass distillation flask, make distinctly acid with  $\text{H}_3\text{PO}_4$ , connect with a condenser, and distil

40–50 cc. With highly colored liquids, make about 200 cc distinctly acid with  $\text{H}_3\text{PO}_4$  and distil as directed previously.

## QUALITATIVE TESTS

19 *I. Phenylhydrazin Hydrochloride Test<sup>1</sup>—Official*

(Gives reliable reactions for  $\text{HCHO}$  in solns varying from 1 part in 50,000 to 1 part in 150,000. Neither acetaldehyde nor benzaldehyde interferes with the reaction.)

With milk and other liquids, shake with an equal volume of 95% alcohol, filter, and use the filtrate. With meats and fats, extract the  $\text{HCHO}$  with alcohol and use the filtrate. With fat, heat the mixture above the melting point of the fat to insure thoro extraction.

Mix 5 cc of the distillate obtained under 18, or of an alcoholic soln or extract, obtained as directed previously, with 0.03 g of phenylhydrazin hydrochloride and 4 or 5 drops of a 1%  $\text{FeCl}_3$  soln. Add slowly and with agitation, in a bath of cold  $\text{H}_2\text{O}$  to prevent heating the liquid, 1–2 cc of  $\text{H}_2\text{SO}_4$ . Dissolve the precipitate by the addition of either  $\text{H}_2\text{SO}_4$  (keeping the mixture cool) or alcohol. In the presence of  $\text{HCHO}$  a red color develops.

20 *II. Hehner Test<sup>2</sup>—Official*

Mix in a test tube about 5 cc of the distillate obtained under 18 with an equal volume of pure milk or with a 1–2% soln of egg albumin, and underlay with commercial  $\text{H}_2\text{SO}_4$  without mixing. A violet or blue color at the junction of the two liquids indicates  $\text{HCHO}$ . This color is given only in the presence of a trace of  $\text{FeCl}_3$  or other oxidizing agent. If only pure acid is available, add a few drops of  $\text{FeCl}_3$  soln to the acid before making the test. Milk may be treated directly by this method, and it gives positive tests in the presence of one or more parts of  $\text{HCHO}$  per 10,000. Other articles of food rich in proteins, for example, egg albumin, give the reaction in the presence of  $\text{H}_2\text{O}$  without the addition of milk.

21 *III. Leach Test —Official*

Mix in a porcelain casserole about 5 cc of the distillate obtained under 18 with an equal volume of pure milk and add about 10 cc of  $\text{HCl}$  soln containing 2 cc of 10%  $\text{FeCl}_3$  soln to each liter of acid. Heat to 80–90° directly over the gas flame, rotating the casserole to break up the curd. A violet coloration indicates  $\text{HCHO}$ .

22 *IV. Phenylhydrazin Hydrochloride and Sodium Nitroprusside Test<sup>3</sup>—Official*

This test may be applied directly to liquid foods, to an aqueous or alcoholic extract of solid foods, or to the distillate prepared as directed under 18. In the case of milk, apply the method directly. With meat, comminute the sample, extract with 2 volumes of hot  $\text{H}_2\text{O}$ , and use the expressed liquid for the test. Heat approximately 10 g of fats above their melting point with 20 cc of alcohol, shake thoroly, cool, filter thru a moistened filter, and use the filtrate for the test.

Dissolve a lump of phenylhydrazin hydrochloride about the size of a pea in 3–5 cc of the liquid to be tested, and add 2–4 drops (not more) of a 5–10% Na nitroprusside soln and 8–12 drops of an approximately 10%  $\text{NaOH}$  soln. If  $\text{HCHO}$  is present, a green or blue color, depending upon the quantity, develops. When  $\text{HCHO}$  is present to the extent of more than 1 part in 70,000–80,000 in the soln tested, a distinct green or bluish green coloration is obtained. In more dilute solns the green tint becomes less marked, and a yellow tinge tending toward greenish brown develops. With this

test acetaldehyde and benzaldehyde give a color varying, according to the strength of the soln, from red to brown. Therefore, a reaction may be obtained with these aldehydes similar to that obtained with HCHO in solns more dilute than 1 part in 70,000. The presence of acetaldehyde or benzaldehyde together with HCHO gives a yellowish or yellowish green tinge. The reaction for HCHO, therefore, may be masked by the presence of other aldehydes, but it is characteristic when a clear green color is obtained.

23 *V. Phenylhydrazin Hydrochloride and Potassium Ferri-  
cyanide Test<sup>b</sup>—Official*

(Not applicable in the presence of the coloring matter of blood.)

Proceed as directed under 22, substituting a soln of  $K_3Fe(CN)_6$  for the Na nitroprusside. HCHO gives a red color. Alcoholic extracts from foods must be diluted with  $H_2O$  to prevent the precipitation of  $K_3Fe(CN)_6$ .

24 *VI. Phenylhydrazin Hydrochloride and Ferric Chloride Test<sup>a</sup>—Official*

Treat 15 cc of milk or other liquid food or of the distillate prepared as directed under 18 with 1 cc of 1% phenylhydrazin hydrochloride soln, then with a few drops of 1%  $FeCl_3$  soln, and finally with HCl. The presence of HCHO is indicated by the formation of a red color, which changes after some time to orange yellow. Milk may be examined directly by this method, but more delicate tests may be obtained from the distillate from milk or from milk serum. Acetaldehyde or benzaldehyde does not interfere with the reaction.

25 *VII. Phloroglucol Test<sup>a</sup>—Official*

To 10 cc of milk or other liquid food under examination in a test tube add, by means of a pipet, 2 cc of phloroglucol reagent (1 g of phloroglucol, 20 g of NaOH, and  $H_2O$  to make 100 cc), placing the end of the pipet on the bottom of the tube in such a manner that the reagent will form a separate layer. If HCHO is present, a bright red coloration (not purple) forms at the zone of contact.

(This soln gives a yellow color in the presence of some aldehydes, and if it is used for the detection of aldehyde formed by the oxidation of methyl alcohol after the destruction of acetaldehyde with  $H_2O_2$  soln, an orange yellow color will slowly appear when an insufficient quantity of  $H_2O_2$  soln has been used. On the other hand, if the excess of  $H_2O_2$  soln is not fully destroyed before the use of this reagent, a purple color develops slowly. The clear red color given by this reagent forms quickly, and in the presence of but a small quantity of HCHO it fades rapidly.)

## SOLUBLE FLUORIDES

26 *QUALITATIVE TESTS<sup>a</sup>—OFFICIAL*

*I. Not Applicable in the Presence of Silicates*

After thoroly mixing the sample transfer to a beaker 150 cc, or an equivalent quantity of an aqueous extract in the case of solid foods, and boil, adding 5 cc of a 10%  $K_2SO_4$  soln and 10 cc of a 10% Ba acetate soln. Collect the precipitate in a compact mass (a centrifuge may be used advantageously) and wash upon a small filter. Transfer to a Pt crucible and ignite.

Dip a carefully cleaned glass plate, while hot, in a mixture of equal parts of carnauba wax and paraffin and allow to cool. Make a distinctive mark thru the wax with a sharp instrument, taking care not to scratch the surface of the glass.

Add a few drops of  $\text{H}_2\text{SO}_4$  to the residue in the crucible and cover the crucible with the waxed plate, having the mark over the center of the crucible and making sure that the edge of the crucible is in close contact with the plate. Keep the top surface of the plate cool by means of a suitable device and heat the crucible for an hour at as high a temp. as practicable without melting the wax (an electric stove gives the most satisfactory form of heat). If fluorides are present, a distinct etching will be apparent on the glass where it was exposed.

27

*II. Applicable in the Presence of Silicates*

Test I may be varied by mixing a small quantity of precipitated  $\text{SiO}_2$  with the precipitated  $\text{BaF}_2$ , and applying the method for the detection of fluosilicates, 29 or 30.

This method is of value in the case of foods, the ash of which contains a considerable quantity of  $\text{SiO}_2$ . Under these circumstances  $\text{H}_2\text{SO}_4$  liberates  $\text{SiF}_4$ , which would escape detection under 26.

**INSOLUBLE FLUORIDES**

28

(Fluorobates, fluosilicates, etc.)

**PREPARATION OF SAMPLE—OFFICIAL**

Make about 200 g of the sample alkaline with lime  $\text{H}_2\text{O}$ , evaporate to dryness, and incinerate. Extract the crude ash with  $\text{H}_2\text{O}$ , to which has been added sufficient acetic acid to decompose carbonates; filter, ignite the insoluble portion, extract with acetic acid (1+2), and again filter. The insoluble portion now contains  $\text{CaSiO}_3$  and  $\text{CaF}_2$ , while the filtrate contains all the  $\text{H}_3\text{BO}_3$  present.

29

**QUALITATIVE TEST I.—OFFICIAL**

Incinerate the filter containing the insoluble portion from 28, mix with a little precipitated  $\text{SiO}_2$ , transfer to a short test tube attached to a small U-tube containing a few drops of  $\text{H}_2\text{O}$ , and add 1-2 cc of  $\text{H}_2\text{SO}_4$ . Keep the test tube in a beaker of  $\text{H}_2\text{O}$  on a steam bath for 30-40 min. If any F is present, the  $\text{SiF}_4$  generated will be decomposed by the  $\text{H}_2\text{O}$  in the U-tube and will form a gelatinous deposit on the walls of the tube.

Next test the filtrate for  $\text{H}_3\text{BO}_3$  as directed under 16. If both HF and  $\text{H}_3\text{BO}_3$  are present, it is probable that they are combined as  $\text{BF}_3$ . If, however,  $\text{SiF}_4$  is detected and  $\text{H}_3\text{BO}_3$  is not, repeat the test without introducing the  $\text{SiO}_2$ , in which case the formation of the silica skeleton is conclusive evidence of the presence of fluosilicate. In an ash containing an appreciable quantity of  $\text{SiO}_2$ ,  $\text{H}_2\text{SO}_4$  will liberate  $\text{SiF}_4$ , rather than HF. Therefore the presence of a fluosilicate, not a fluoride, is indicated.

30

**QUALITATIVE TEST II.—OFFICIAL**

Incinerate the filter containing the insoluble portion from 28 in a Pt crucible, mix with a little precipitated  $\text{SiO}_2$ , and add 1 cc of  $\text{H}_2\text{SO}_4$ . Cover the crucible with a watch-glass from the underside of which a drop of  $\text{H}_2\text{O}$  is suspended, and heat for an hour at 70-80°, keeping the watch-glass well cooled. The  $\text{H}_2\text{O}$  decomposes the  $\text{SiF}_4$  which is formed, leaving a gelatinous deposit of  $\text{SiO}_2$  and etching a ring at the periphery of the drop of  $\text{H}_2\text{O}$ . Test the filtrate for  $\text{H}_3\text{BO}_3$  as directed under 16.

**SULFUROUS ACID**

31

*Qualitative Test<sup>1</sup>—Official*

Add a small quantity of S-free zinc and several cc of HCl to about 25 g of the sample (with the addition of  $\text{H}_2\text{O}$ , if necessary) in a 200 cc Erlenmeyer flask. The

$\text{H}_2\text{S}$  generated in the presence of sulfites may be detected with Pb acetate paper. The traces of metallic sulfides occasionally present in vegetables will give the same reaction as sulfites under the conditions of the above test. Verify positive results obtained by this method by the Monier-Williams method, 32.

It is always advisable to make the quantitative determination of sulfites, owing to the danger that the test may be caused by traces of sulfides. A trace should not be considered sufficient indication of the presence of  $\text{SO}_2$  either as a bleaching agent or as a preservative.

#### TOTAL SULFUROUS ACID

32

##### *Monier-Williams Method*<sup>12</sup>—Official

(Applicable in presence of other volatile sulfur compounds.)

Connect a 750 cc round-bottomed Pyrex flask (B) (Fig. 40) to a sloping reflux condenser (D), the lower end of which is cut off at an angle. (Monier-Williams pre-

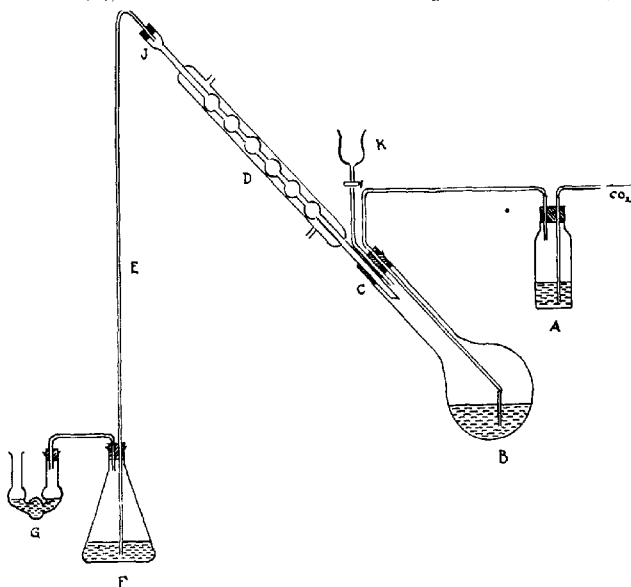


FIG. 40.—MONIER-WILLIAMS APPARATUS FOR DETERMINATION OF SULFUROUS ACID

fers using an upright round-bottomed flask with 2 necks.) Pass  $\text{CO}_2$  from a generator thru a  $\text{Na}_2\text{CO}_3$  soln in A to remove Cl. Also connect a dropping funnel (K) to B by the three-holed stopper C. Use the tube E to connect the upper end of the condenser to a 200 cc Erlenmeyer flask (F), which is followed by a Peligot tube (G). This delivery tube (E) extends to the bottom of the receiver. One Peligot tube has been

found to be sufficient to catch traces of sulfurous acid swept thru the flask F. Use rubber stoppers thruout. The receiver F contains 15 cc of pure neutral 3%  $\text{H}_2\text{O}_2$ , while the Peligot tube contains 5 cc.  $\text{H}_2\text{O}_2$  usually contains free  $\text{H}_2\text{SO}_4$ . Start with 30%  $\text{H}_2\text{O}_2$ , dilute somewhat, and neutralize with  $\text{Ba}(\text{OH})_2$  soln, using bromophenol blue soln as indicator. After the reagent has settled in the cold, filter from the  $\text{BaSO}_4$ , determine its strength by permanganate titration, and finally adjust to a 3% strength. The bromophenol blue indicator in the  $\text{H}_2\text{O}_2$  remains unaffected for some time.

After connecting the apparatus, introduce into the flask 300 cc of distilled  $\text{H}_2\text{O}$  and 20 cc of  $\text{HCl}$  and boil for a short time in a current of  $\text{CO}_2$ . Then add the food to be tested, adopting the procedure to the sort of food. Add liquids directly by means of the dropping funnel. In the case of easily transferable solids, first cool the contents of the flask somewhat, taking care to regulate the flow of  $\text{CO}_2$  to avoid having the  $\text{H}_2\text{O}_2$  drawn up in the delivery tube E. Then quickly introduce the food by removing the stopper C. With semi-solid foods, requiring more time to introduce into the flask, cool the flask contents by gradual immersion in cold  $\text{H}_2\text{O}$ , and wash the food in quickly with recently boiled distilled  $\text{H}_2\text{O}$ . After introducing the food, boil the mixture for 1 hour ( $1\frac{1}{2}$  hours in the case of dried fruits) in a slow current of  $\text{CO}_2$ , stopping the flow of  $\text{H}_2\text{O}$  in the condenser just before the end of the distillation. This causes the condenser to become hot and drives over residual traces of  $\text{SO}_2$  retained in the condenser. When the delivery tube just above the receiver E becomes hot to the touch, remove stopper J immediately.

Wash the delivery tube and the Peligot tube contents into the flask F, and titrate the liquid at room temp. with 0.1 N  $\text{NaOH}$ , using bromophenol blue as indicator. The  $\text{NaOH}$  must be standardized with this indicator. Bromophenol blue is unaffected by  $\text{CO}_2$  and also gives a distinct color change in cold  $\text{H}_2\text{O}_2$ . 1 cc of 0.1 N  $\text{NaOH}$  = 3.2 mg of  $\text{SO}_2$ , so that titration of small quantities of  $\text{SO}_2$  requiring less than 0.5 cc of  $\text{NaOH}$  is not accurate. A gravimetric determination may be made after titration, the precipitation of  $\text{BaSO}_4$  being carried out at room temp. After allowing the supernatant liquid to settle, filter, and wash the residual  $\text{BaSO}_4$  3 times by decantation with boiling  $\text{H}_2\text{O}$ . Determine a blank on the reagents, both by titration and gravimetrically, and correct the results accordingly.

33

**FREE SULFUROUS ACID—OFFICIAL**

(Especially adapted to wine.)

Treat 50 cc of the sample in a 200 cc flask with about 5 cc of  $\text{H}_2\text{SO}_4$  (1+3), add about 0.5 g of  $\text{Na}_2\text{CO}_3$  to expel the air, and titrate the sulfurous acid with 0.02 N I soln, using a few cc of starch indicator, VI, 3(e). Introduce the I soln as rapidly as possible and continue the addition until the blue color persists for several min. 1 cc of 0.02 N I = 0.64 mg of  $\text{SO}_2$ .

**BETA-NAPHTHOL**

34

**QUALITATIVE TEST—TENTATIVE**

In a separatory funnel extract 200 cc of the sample or of its aqueous extract, prepared as directed under 1(c), with 10 cc of  $\text{CHCl}_3$ . To the  $\text{CHCl}_3$  extract in a test tube add a few drops of 0.5 N  $\text{KOH}$  soln and place in a boiling water bath for 2 min. The presence of beta-naphthol is indicated by the formation of a deep blue color which changes to green and then to yellow.

## ABRASTOL (ASAPROL)

35

I. SINIBALDI METHOD<sup>14</sup>—TENTATIVE

Make 50 cc of the sample alkaline with a few drops of  $\text{NH}_4\text{OH}$  and extract with 10 cc of amyl alcohol, adding ethyl alcohol if an emulsion forms. Decant the amyl alcohol, filter if turbid, and evaporate to dryness. Add to the residue 2 cc of  $\text{HNO}_3$  (1+1), heat on a water bath until half of the liquid is evaporated, and transfer to a test tube with the addition of 1 cc of  $\text{H}_2\text{O}$ . Add about 0.2 g of crystallized  $\text{FeSO}_4$  and an excess of  $\text{NH}_4\text{OH}$ , dropwise, with constant shaking. If the resultant precipitate is of a reddish color, dissolve it in a few drops of  $\text{H}_2\text{SO}_4$  and add crystallized  $\text{FeSO}_4$  and  $\text{NH}_4\text{OH}$  as before. As soon as a dark colored or greenish precipitate is obtained, introduce 5 cc of 95% alcohol, dissolve the precipitate in  $\text{H}_2\text{SO}_4$ , shake well, and filter. In the absence of abrastol a colorless or light yellow liquid is produced, while a red color is produced in the presence of 0.01 g of abrastol.

36

II. SANGLÉ-FERRIÈRE METHOD<sup>15</sup>—TENTATIVE

Boil 200 cc of the sample with 8 cc of  $\text{HCl}$  for an hour in a flask fitted with a reflux condenser. Abrastol is thus converted into beta-naphthol and is detected as directed under 34.

## SUCROL OR DULCIN

37

I. MORPURGO METHOD<sup>16</sup>—TENTATIVE

Evaporate about 100 cc of the sample, or of the aqueous extract prepared as directed under 1(c) and neutralized with acetic acid, to a sirupy consistency after the addition of about 5 g of basic  $\text{PbCO}_3$ , and extract the residue several times with 90% alcohol. Evaporate the alcoholic extract to dryness, extract the residue with ether, and allow the ether to evaporate spontaneously in a porcelain dish. Add 2 or 3 drops each of phenol and  $\text{H}_2\text{SO}_4$  and heat for about 5 min. on a water bath. Cool, transfer to a test tube, and overlay with  $\text{NH}_4\text{OH}$  or  $\text{NaOH}$  soln with the least possible mixing. The presence of dulcin is indicated by the formation of a blue color at the zone of contact.

38

II. JORISSEN METHOD<sup>17</sup>—TENTATIVE

Suspend the residue from the ether extract obtained as directed under 37 in about 5 cc of  $\text{H}_2\text{O}$ , add 2-4 cc of an approximately 10% soln of  $\text{Hg}(\text{NO}_3)_2$ , and heat for 5-10 min. on a steam bath. In the presence of sucrol a violet blue color is formed. On the addition of  $\text{PbO}_2$  the color changes to a deep violet.

FORMIC ACID<sup>18</sup>—OFFICIAL

39

## REAGENTS

(a) *Sodium acetate*.—Dissolve 50 g of dry Na acetate in sufficient  $\text{H}_2\text{O}$  to make 100 cc and filter.

(b) *Mercuric chloride*.—Dissolve 100 g of  $\text{HgCl}_2$  and 150 g of  $\text{NaCl}$  in sufficient  $\text{H}_2\text{O}$  to make 1 liter and filter.

40

## APPARATUS

The apparatus required (Fig. 41) consists of a steam generator (S), a 300 cc flask (A) in which the sample is placed, a 500 cc flask (B) containing a suspension of  $\text{BaCO}_3$ , a spray trap (T), a condenser, and a 1 liter volumetric flask (C). The tip of the tube D, leading into B, consists of a bulb containing a number of small holes to break the vapor into small bubbles.

41

## DETERMINATION

Use 50 cc of thin liquids like fruit juices; for heavy liquids and semi-solids like sirups and jams, use 50 g diluted with 50 cc of  $H_2O$ . Place the sample in flask A, add 1 g of tartaric acid, and connect as shown in Fig. 41, the flask B having been charged previously with a suspension of 2 g of  $BaCO_3$  in 100 cc of  $H_2O$ . If much acetic acid is present, use sufficient  $BaCO_3$  so that at least 1 g remains at the end of the operation. Heat the contents of flasks A and B to boiling and distil with steam from the generator S, the vapor passing first thru the sample in flask A, then thru the boiling suspension of  $BaCO_3$  in B, after which it is condensed and collected in the volumetric

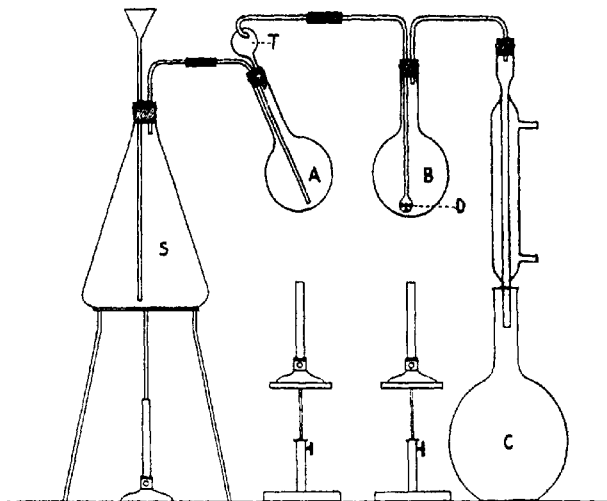


FIG. 41.—APPARATUS FOR DETERMINATION OF FORMIC ACID

flask C. Continue the distillation until 1 liter of distillate is collected, maintaining the volume of the liquids in the flasks A and B as nearly constant as possible by heating with small Bunsen flames and avoiding charring of the sample in the flask A. After collecting 1 liter of distillate, disconnect the apparatus and filter the contents of flask B while hot, washing the  $BaCO_3$  with a little hot  $H_2O$ . The filtrate and washings should now measure about 150 cc; if they do not, they should be boiled down to that volume. Add 10 cc of the Na acetate soln, 2 cc of a 10% soln of HCl, and 25 cc of the  $HgCl_2$ . Mix thoroly and immerse the container in a boiling water or steam bath for 2 hours. Filter thru a weighed Goocherueible and wash the precipitate thoroly with cold  $H_2O$  and finally with a little alcohol. Dry in a boiling water oven for 30 min., cool, weigh, and calculate the weight of  $HCOOH$  present by multiplying the weight of the precipitate by 0.0975. If the weight of  $HgCl$  obtained exceeds 1.5 g, repeat the determination, using more  $HgCl_2$  or a smaller quantity of sample. Conduct a blank test with each new lot of reagents employed for the reduction,



using 150 cc of  $H_2O$ , 1 cc of 10%  $BaCl_2$  soln, 2 cc of the  $HCl$  soln, 10 cc of the  $Na$  acetate, and 25 cc of the  $HgCl_2$ , and heating the mixture in a boiling water or steam bath for 2 hours. Deduct the weight of  $HgCl$  obtained in this blank test from that obtained in the regular determination.

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### XXXIII. SPICES AND OTHER CONDIMENTS

#### SPICES

1 **PREPARATION OF SAMPLE—OFFICIAL**

Grind the sample to pass thru a sieve having circular openings 1 mm in diameter and mix thoroly. Owing to the lack of uniformity of most spices and the peculiar tendency to stratify, use extreme care in weighing out a portion for analysis. Stir the material thoroly and weigh out a 2 g sample, using a spoon with a capacity of about 2 g. Dip a spoonful from the center of the material, being careful to take approximately the required quantity so as to avoid adding to or taking from the portion on the scale pan. In the determination of starch in spices by the diastase method, further reduce a subsample as nearly as possible to an impalpable powder.

2 **MOISTURE—TENTATIVE**

Dry 2 g to constant weight at 110°. From the resulting loss in weight subtract the quantity of volatile ether extract as determined under 9.

3 **ASH—OFFICIAL**

Proceed as directed under XXVII, 8.

4 **SOLUBLE AND INSOLUBLE ASH—OFFICIAL**

Proceed as directed under XXXIV, 12, using the ash obtained under 3.

5 **ASH INSOLUBLE IN ACID—OFFICIAL**

Boil the water-insoluble residue obtained under 4, or the total ash obtained as directed under 3, with 25 cc of HCl (1+2.5) for 5 min., collect the insoluble matter on a Gooch crucible or on an ashless filter, wash with hot H<sub>2</sub>O, ignite, cool, and weigh.

6 **CALCIUM OXIDE IN ASH—OFFICIAL**

Ignite 2-4 g of the sample as directed under 3, digest with hot HCl (1+2.5), evaporate to dryness, moisten the dry residue with the dilute HCl, and again evaporate to dryness to render the SiO<sub>2</sub> insoluble. Treat the residue with 5-10 cc of HCl, add about 50 cc of H<sub>2</sub>O, allow to stand on a water bath for a few minutes, filter, and wash the insoluble residue with hot H<sub>2</sub>O. Determine CaO in the combined filtrate and washings as directed under XII, 10.

7 **NITROGEN—OFFICIAL**

Proceed as directed under II, 21, 23 or 25, except in the case of black and white peppers, for which use only the Kjeldahl-Gunning-Arnold method<sup>1</sup> (II, 25), using 1 g of the sample.

8 **NITROGEN IN NON-VOLATILE ETHER EXTRACT—OFFICIAL**

(For black and white peppers.)

Extract 10 g of the pepper for 20 hours in a continuous extraction apparatus with absolute ether, collecting the extract in a weighed 250 cc flask. Evaporate the ether and dry first at 100° and finally to constant weight at 110°. Determine the N in the weighed extract as directed under II, 25, digesting in the same flask used for the

extraction. Calculate the parts of N per 100 parts of non-volatile ether extract. If desired, crude piperine may be calculated from the N by multiplying by 20.36.

9 VOLATILE AND NON-VOLATILE ETHER EXTRACT<sup>2</sup>—OFFICIAL

Extract 2 g of the ground material for 20 hours in a continuous extraction apparatus with anhydrous ether (XXVII, 22). Transfer the ethereal soln to a weighed capsule and allow to evaporate at room temp. Let stand for 18 hours over H<sub>2</sub>SO<sub>4</sub>, and weigh the total ether extract. Heat the extract gradually and then to constant weight at 110°. The loss is volatile ether extract; the residue is non-volatile ether extract.

10 ALCOHOL EXTRACT<sup>2</sup>—OFFICIAL

Place 2 g of the sample in a 100 cc flask and fill to the mark with 95% alcohol. Stopper, shake for 8 hours at 30 min. intervals, and allow to stand for 16 hours longer without shaking. Filter the extract thru a dry filter, evaporate a 50 cc aliquot of the filtrate to dryness in a flat-bottomed dish on a steam bath, and heat to constant weight at 110°.

11 COLD-WATER EXTRACT—TENTATIVE

(For ginger.)

Place 4 g of the sample in a 200 cc volumetric flask, add H<sub>2</sub>O to the mark, shake at 30 min. intervals during 8 hours, and let stand 16 hours longer without shaking. Filter and evaporate a 50 cc aliquot of the filtrate to dryness in a flat-bottomed metal dish. Dry to constant weight at 100°.

12 COPPER-REDUCING SUBSTANCES BY DIRECT INVERSION—OFFICIAL

Extract 4 g of the sample with 5 successive portions of 10 cc of ether on a filter that will retain completely the smallest starch granules. After the ether has evaporated, wash with 150 cc of alcohol, 10% by volume.

Owing to the formation of a glutinous mass, which clogs the filter, it is not possible to wash samples of Batavia cassia with H<sub>2</sub>O or dilute alcohol. Therefore it is best to omit all preliminary washing in determinations made on all varieties of cassia, as well as on cassia buds and cinnamon.

Carefully wash the residue from the paper into a 500 cc flask with 200 cc of H<sub>2</sub>O, using a small wash bottle and gently rubbing the paper with the tip of the finger. Hydrolyze and determine the Cu reducing material as directed under XXVII, 31. Express the result in terms of starch.

13 STARCH—OFFICIAL

Extract 4 g of the finely pulverized sample with ether and 500 cc of 10% alcohol, as directed under 12, and determine starch by the diastase method, as directed under XXVII, 33.

14 CRUDE FIBER—OFFICIAL

Proceed as directed under XXVII, 27, and previous to weighing remove all ether extractives by successive washings of the dry fiber with ether.

15 TANNIN—OFFICIAL

(For cloves and allspice.)

Extract 2 g of the sample for 20 hours with anhydrous ether. Boil the residue for 2 hours with 300 cc of H<sub>2</sub>O, cool, make up to 500 cc, and filter. Measure 25 cc of this

infusion into a 2 liter porcelain dish, add 20 cc of indigo soln, XV, 29(c), and 750 cc of  $H_2O$ , and proceed as directed under XV, 30. 1 cc of 0.1 *N* oxalic acid = 0.00623 g of quercitannic acid, or 0.0008 g of O absorbed.

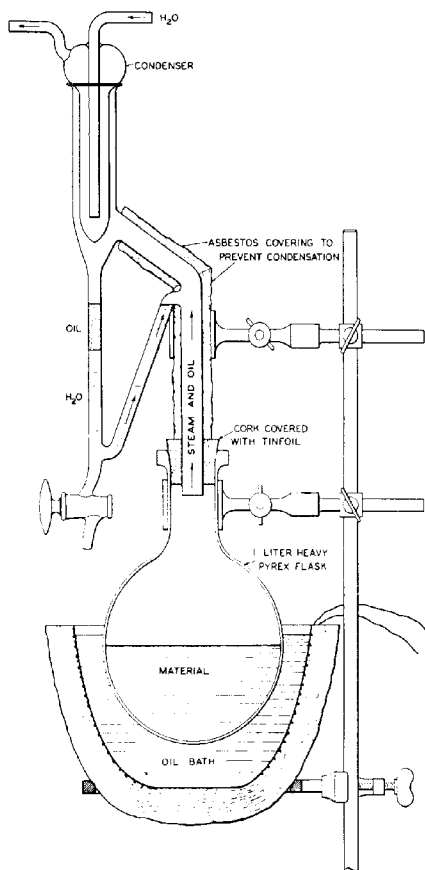


FIG. 42.—APPARATUS FOR THE DETERMINATION OF VOLATILE OIL

16

#### VOLATILE OIL—TENTATIVE

Transfer a weighed quantity of whole or ground material to a 500–2000 cc round-bottomed, short-necked flask in amount sufficient to yield, if possible, 2 cc or more

of volatile oil. Add to the flask 3-6 times as much  $H_2O$  as material and mix uniformly. Set up the apparatus, Fig. 42, using the appropriate volatile oil trap illustrated in Fig. 43. With an oil bath (hydrogenated cottonseed oil is satisfactory) as the source of heat, boil the contents of the flask slowly 4-8 hours, or until all the volatile oil has been distilled, taking care to avoid the escape of vapors around the condenser. With spices (for example nutmeg) containing volatile oils lighter than  $H_2O$  and also fixed oils heavier than  $H_2O$ , discontinue the distillation when the fraction of oil obtained during a 1 hour period is heavier than  $H_2O$ .

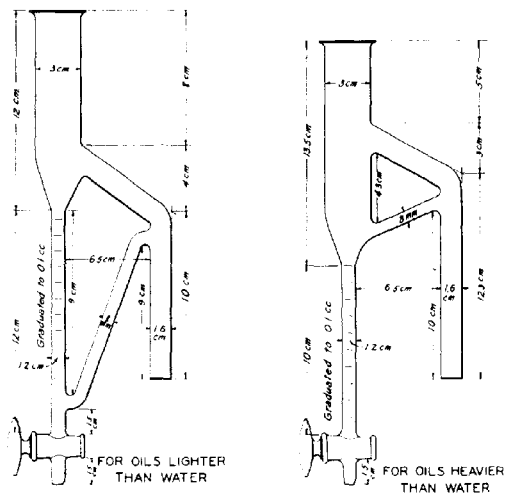


FIG. 43.—TYPES OF OIL SEPARATORY TRAPS

In case of unsatisfactory separation of the volatile oil, draw off the contents of the trap into a small separatory funnel. After separation return the  $H_2O$  to the trap and transfer the volatile oil to a graduated cylinder. Repeat the procedure if necessary.

With volatile oils heavier than  $H_2O$ , after transferring to the graduated cylinder run the  $H_2O$  with any remaining oil into a small separatory funnel. Wash the oil trap with 10 cc of ether and transfer the washings to the funnel. Shake, and withdraw the ether. Evaporate the ether and drain the residue into the cylinder. Read the quantity of volatile oil directly in the cylinder and report the oil in terms of cc per 100 g of spice.

Determine the physical and appropriate chemical characteristics of the volatile oil as directed under 17-23, after it has been allowed to stand until perfectly clear or been dried with a minimum quantity of anhydrous  $Na_2SO_4$  and allowed to settle. (Owing to the small quantity of oil available great care should be exercised to avoid loss in any of these determinations.)

**17 SPECIFIC GRAVITY OF VOLATILE OIL—TENTATIVE**

Determine sp. gr. at 25/25° as directed under XIV, 3, using a 1 cc Sprengel tube.

**18 OPTICAL ROTATION OF VOLATILE OIL—TENTATIVE**

Polarize in a micropolarizing tube 50 mm long and of approximately 2 mm bore, with white light at 25°. (The tube may be readily filled by the aid of a glass tube drawn out to a diameter smaller than the bore of the tube.) Report in angular degrees on the basis of a 100 mm tube.

**19 REFRACTIVE INDEX OF VOLATILE OIL—OFFICIAL**

Proceed as directed under XXXI, 8 and 9.

**20 ACID NUMBER OF VOLATILE OIL—TENTATIVE**

Add 30 cc of neutral alcohol to approximately 2 g of the volatile oil, accurately weighed, in a 200 cc Erlenmeyer flask. Titrate with 0.1 N KOH, using 1–2 drops of 1% phenolphthalein as an indicator.

$$\text{Acid No.} = \frac{\text{cc } 0.1 \text{ N KOH} \times 5.61}{\text{Wt. of volatile oil}}$$

**21 ESTER NUMBER OF VOLATILE OIL—TENTATIVE**

To the contents of the flask after determination 20, add exactly 20 cc of 0.5 N KOH. Heat the flask on a water bath approximately 2 hours, using an air condenser 70–80 cm long and 5–8 mm in diameter. Determine the cc of 0.5 N KOH used in the saponification (a) by titrating the excess with 0.5 N H<sub>2</sub>SO<sub>4</sub>, using 1–2 drops of phenolphthalein as an indicator.

$$\text{Ester number} = \frac{(a) \times 28.06}{\text{Wt. of volatile oil}}$$

**22 EUGENOL IN VOLATILE OIL—TENTATIVE**

Measure 2 cc of volatile oil (transfer pipet) into a Babcock milk bottle, XXII, 20(a). Add 20 cc of 3% soln of KOH, shake the mixture 5 min., heat for 10 min. in a boiling water bath, remove, and cool to room temp. When the liquids have separated completely, add sufficient KOH soln to bring the residual oil within the graduated portion of the neck and note the volume. Calculate the percentage by volume of eugenol from the difference of volume of the sample used and the residual oil.

**23 KETONE AND ALDEHYDE IN VOLATILE OIL—TENTATIVE**

Measure 2 cc of volatile oil (transfer pipet) into a Babcock milk bottle, XXII, 20(a). Add 10 cc of a saturated soln of Na<sub>2</sub>SO<sub>3</sub> and a few drops of 1% phenolphthalein. Heat in a bath containing boiling H<sub>2</sub>O, and shake the flask repeatedly, neutralizing the mixture occasionally with a few drops of a saturated soln of NaHSO<sub>3</sub>. If no coloration appears upon adding a few drops of phenolphthalein and heating for 30 min., cool to room temp. When the liquids have separated completely, add sufficient Na<sub>2</sub>SO<sub>3</sub> soln to bring the residual oil within the graduated portion of the neck and note the volume. Calculate the percentage by volume from the difference of volume of the sample used and the residual oil.

**24 VOLATILE OIL AND RESIN IN GINGER<sup>4</sup>—TENTATIVE**

Place 50 g of ground ginger in a Soxhlet extractor and extract completely, using ether as the solvent (about 4 hours). Transfer the extract to a 300 cc flask and evaporate off the ether on the steam bath until the solvent is no longer detected. Add 50 cc of  $H_2O$  to the residue and determine the yield of volatile oil (using trap for oils lighter than  $H_2O$ ) and determine sp. gr., optical rotation, refractive index, acid and ester numbers as directed under 17–21.

Transfer the residue in the flask to a separatory funnel and extract the resin with ether. Transfer to a tared beaker, evaporate the ether on a steam bath, and dry to constant weight in a vacuum desiccator and calculate to percentage.

**25 VOLATILE OIL IN MUSTARD SEED<sup>7</sup>—OFFICIAL**

Place 5 g of the ground seed (No. 20 powder) in a 200 cc flask, add 100 cc of  $H_2O$ , stopper tightly, and macerate for 2 hours at about 37°. Then add 20 cc of 95% alcohol and distil about 60 cc into a 100 cc volumetric flask containing 10 cc of  $NH_4OH$  (1+2), taking care that the end of the condenser dips below the surface of the soln. Add 20 cc of 0.1 N  $AgNO_3$  soln to the distillate, set aside overnight, heat to boiling on a water bath in order to agglomerate the  $(Ag)_2S$ , cool, make up to 100 cc with  $H_2O$ , and filter. Acidify 50 cc of the filtrate with about 5 cc of  $HNO_3$  and titrate with 0.1 N  $NH_4CNS$ , using 5 cc of 10% ferric ammonium sulfate soln as an indicator. 1 cc of 0.1 N  $AgNO_3$  consumed = 0.004956 g of allyl isothiocyanate.

**IODINE NUMBER OF PAPRIKA OIL<sup>8</sup>**

(Indicative of presence of olive oil.)

**26 Qualitative Test—Tentative**

Transfer 10 g of the well-mixed ground sample to a 200 cc glass-stoppered flask and add 100 cc of  $CHCl_3$  from a pipet, rotating while adding the first 50 cc. Let stand 1 hour, shake, and filter thru a 12½ cm fluted filter. Pipet off successively two 10 cc portions, using the same pipet. Transfer one of the 10 cc portions to a weighed crystallizing dish, 50 × 35 mm, and evaporate the solvent by placing the dish on a steam bath. Dry the dish and contents at 100° for 1 hour, cool in air, and weigh. Use the weight obtained in calculating the I number. Transfer the other 10 cc portion to a suitable glass-stoppered flask or bottle for the determination of the I number, proceeding as directed under XXXI, 19, allowing 30 min. for the halogen absorption. Calculate the I number of the ether extract. The I number of pure paprika thus obtained should be not less than 125.

**MICROSCOPIC EXAMINATION—TENTATIVE****27 GENERAL**

Adulterants of vegetable origin in spices are best detected by means of the microscope. A general knowledge of vegetable histology and the microscopic appearance of the spices and spice adulterants is essential. Some of the standard works<sup>9</sup> on these subjects are listed under the selected references.

**28 REAGENTS**

(a) *Ammoniacal copper soln (Schweitzer's reagent).*—Add slowly a soln of  $CuSO_4$  to a soln of  $NaOH$ , separate by filtration the precipitate of  $Cu(OH)_2$  that forms and

wash it thoroly with  $H_2O$ . Dissolve the wet precipitate in  $NH_4OH$  with the aid of heat, cool, and filter. Prepare immediately before use and keep in the dark.

(b) *Potassium hydroxide soln.*—Dissolve 5 g of KOH in  $H_2O$  and dilute to 100 cc.

(c) *Chloral hydrate.*—Dissolve 8 parts by weight of the crystals in 5 parts of  $H_2O$ .

(d) *Acidified chloral hydrate-glycerol soln.*—Dissolve 45 g of crystals of chloral hydrate in 25 cc of HCl (1+8) and 10 cc of glycerol.

(e) *Schultze's mixture.*—Mix crystallized  $KClO_3$  with  $HNO_3$  as needed.

(f) *Iodine-potassium iodide soln (iodine soln).*—Dissolve 0.05 g of I and 0.2 g of KI in 15 cc of  $H_2O$ .

(g) *Chlor-zinc iodine soln.*—Dissolve 100 g of  $ZnCl_2$  in 60 cc of  $H_2O$  in a glass-stoppered bottle and add 20 g of KI and 0.5 g of I crystals. Leave a few crystals of I in the bottle to insure saturation, and allow the soln to stand a few hours before using. (This soln will keep for months. If the color developed in the tissue is too deep a blue, a very slight dilution of the reagent is advisable.)

(h) *Ferric acetate or chloride soln.*—Use a freshly prepared 1% aqueous soln.

(i) *Alkanet tincture.*—Macerate 20 g of alkanet root for several days with 100 cc of 95% alcohol.

(j) *Safranine soln.*—Prepare a saturated aqueous soln and dilute as needed.

(k) *Mayer's reagent (mercuric-potassium iodide soln).*—Dissolve 1.36 g of  $HgCl_2$  in 60 cc of  $H_2O$  and 5 g of KI in 10 cc of  $H_2O$ ; mix these two solns and dilute to 100 cc.

## 29

## APPARATUS

(a) *Dissecting microscope or hand lens.*

(b) *Compound microscope.*—Provided with objectives and oculars capable of giving a range of about 4 different magnifications varying from 75–500 diameters, a double or triple nosepiece, and a substage condenser. An eyepiece micrometer, a polarizing apparatus, and a mechanical stage are desirable for some special types of work.

(c) *Sieves.*—A series of standard mesh sieves varying from 10 to 100 meshes per inch and a sieve having circular openings 1 mm in diameter.

(d) *Slides, cover glasses, needles, forceps, etc.*

## 30

## PREPARATION OF SAMPLE

Reduce one portion to a fine powder in a mortar. Separate another portion into several grades of fineness by sieves of different mesh or by jarring on a sheet of paper. In the coarser grades fragments of suspicious nature may often be seen with the naked eye or under a simple microscope; these should be picked out for subsequent examination under the compound microscope.

## 31

## EXAMINATION

Mount a small quantity of the ground sample in  $H_2O$  and examine under the compound microscope with both ordinary and polarized light. This gives general information as to the nature of the material and serves for the detection and identification of starch granules and various tissues. Place a small drop of the I-KI soln at the edge of the cover-glass, draw it into the preparation by means of a piece of filter paper placed at the opposite edge of the cover-glass, and examine again. Starch granules will be colored blue or blue-black; cellulose, yellow; and proteins, either brown or yellow.



In the manner just described draw a little of the KOH soln under the cover-glass and again examine. This treatment gelatinizes the starch granules, dissolves the proteins, saponifies the fats, and in other ways clears the preparation. It also imparts to tannins a reddish color. If this treatment does not clear the tissues satisfactorily, treat a fresh portion for a short time with the acidified chloral hydrate-glycerol soln, or for some hours with the chloral hydrate soln.

Examine also the crude fiber obtained in the chemical analysis, as in this material the stone cells and other tissues are shown distinctly.

To isolate stone cells, bast fibers, and other thick-walled cells, macerate a portion of the sample in Schultze's mixture, using such proportions of  $\text{KClO}_3$  and  $\text{HNO}_3$  and heating for such a time as will secure the desired results.

To distinguish cellulose from infiltrated substances (lignin, suberin, etc.), add freshly prepared chlor-zinc iodine soln to a water mount. Cellulose is colored blue, and infiltrated substances are yellow.

To distinguish fats, oils, essential oils, and resins from other cell contents, treat for an hour with alkanet tincture diluted with an equal volume of  $\text{H}_2\text{O}$ , which imparts to these substances a deep red color, or treat with ether, which dissolves them. Treat also with alcohol, which dissolves the essential oils and resins but does not perceptibly affect the fats and oils.

Test for proteins by warming cautiously on a slide with a drop of freshly prepared Millon's reagent. The proteins are partially decomposed, acquiring gradually a brick red color. If it is desired to study the form of the aleurone (protein) granules, which in some plants are quite as characteristic as starch granules, prepare a mount in pure glycerol or oil.

Test for tannins and tissues impregnated with them by adding 1% ferric acetate or chloride soln. Both of these reagents give a green or blue color with tannins, but the ferric acetate acts more slowly and is to be preferred.

Crystals of Ca oxalate<sup>9</sup> are recognized by their characteristic forms and by their behavior to polarized light. To distinguish Ca oxalate from  $\text{CaCO}_3$ , treat with acetic acid, which does not affect the oxalate but dissolves the carbonate with effervescence. Both are soluble in  $\text{HCl}$ .

Powdered charcoal and charred shells resist the bleaching action of potash, chloral hydrate, and Schultze's mixture.

### PREPARED MUSTARD

32

#### PREPARATION OF SAMPLE—OFFICIAL

Transfer the entire contents of the container to a dish sufficiently large to permit thoro stirring and make the whole mass homogeneous. Preserve in a bottle having a tightly fitting glass stopper. Stir well each time before removing a portion for analysis.

33

#### SOLIDS—OFFICIAL

Weigh 5 g of the sample into a flat-bottomed Pt dish; distribute evenly over the bottom of the dish with a little  $\text{H}_2\text{O}$ , place on a steam bath until the mixture appears dry, and then heat in an oven at  $100^\circ$  to constant weight.

34

#### ASH—OFFICIAL

Ignite the dry residue, obtained in the determination of solids, 33, as directed under XXVII, 8.

35

## SALT—OFFICIAL

Determine Cl in the ash as directed under XII, 35 or 37.

36

## ETHER EXTRACT—TENTATIVE

Weigh 10 g of the sample into any ordinary Si, Al or porcelain drying dish and mix with about 30 g of sand. Heat on a water bath until the mixture appears dry and complete the drying in a water oven. Grind until all the lumps are broken up, and determine the ether extract as directed under XXVII, 22.

37

## PROTEIN—OFFICIAL

Determine the N as directed under II, 21, 23, or 25, using 5 g of the sample. Multiply the result by 6.25 to obtain the equivalent quantity of protein.

38

## ACIDITY—OFFICIAL

Weigh 10 g of the sample into a 200 cc volumetric flask, dilute to the mark with  $H_2O$ , shake, filter thru a dry paper, and determine the acidity in 100 cc by titration with 0.1 N alkali, using phenolphthalein indicator. Express the result as acetic acid. 1 cc of 0.1 N alkali = 0.0060 g of acetic acid.

39

## COPPER-REDUCING SUBSTANCES BY DIRECT INVERSION—OFFICIAL

Proceed as directed under XXVII, 31, except to treat directly 10 g of the sample, without previous washing or extraction, with 200 cc of  $H_2O$  and 20 cc of HCl (sp. gr. 1.125), and to make up the soln to 250 cc after neutralizing and before filtering and drawing off the aliquot. In analyses of samples containing starch, particular attention should be given that the quantity of dextrose present in the aliquot taken for the reducing sugar determination does not exceed the maximum permitted for that determination. Express the result in terms of starch.

40

CRUDE FIBER<sup>11</sup>—OFFICIAL

Weigh 10 g of the sample and transfer to an 8 oz nursing bottle with 50 cc of 95% alcohol, stopper, and shake vigorously. Add 40 cc of ether, shake, and let stand about 5 min. with occasional shaking. Centrifuge and decant the alcohol-ether mixture. Treat twice more with 40 cc portions of ether, shaking, centrifuging, and decanting as before. Rest the bottle on its side for a short time, without heat, to allow most of the ether to evaporate. Transfer the material to a 500 cc Erlenmeyer flask, using the 200 cc of boiling  $H_2SO_4$ , XXVII, 25(a), and proceed as directed under XXVII, 27, but in addition wash the fiber with successive portions of ether previous to drying and weighing.

If preferred, the sample may be treated with alcohol and ether in a small beaker, transferred to a hardened 11 cm filter paper, washed several times with ether, and transferred to the 500 cc Erlenmeyer flask with the 200 cc of boiling hot dilute  $H_2SO_4$ .

41

## COLORING MATTER—TENTATIVE

Proceed as directed under XXI.

42

## PRESERVATIVES—OFFICIAL

Proceed as directed under XXXII.

**SALAD DRESSINGS<sup>1</sup>**

43

**PREPARATION OF SAMPLE—TENTATIVE**

Before removing any portion of the sample for analysis and each time a subsequent portion is removed, if the sample has stood for any appreciable length of time, mix until it is homogeneous. For the various determinations, take approximately the quantity directed and weigh. (A Bailey weighing buret<sup>13</sup> will be found convenient.)

44

**TOTAL SOLIDS<sup>14</sup>—OFFICIAL**

Use a 2 g sample and proceed as directed in **XXIII, 2**.

45

**REDUCING SUGARS BEFORE INVERSION—TENTATIVE**

Weigh 20 g of the sample into a wide-mouthed, 4 oz bottle and extract the oil by adding about 80 cc of petroleum ether, shaking, and centrifuging. Draw off as much as possible of the petroleum ether soln (conveniently done by using suction and a short-stemmed pipet), and repeat the treatment with petroleum ether until all the oil has been removed. This is indicated by the absence of color in the solvent. Usually about four extractions are required. Reserve the ether soln for identification of the oil. Remove the petroleum ether from the residue with a current of air and transfer the residue with H<sub>2</sub>O to a 100 cc volumetric flask. Add 5–10 cc of a fresh soln of HPO<sub>3</sub> (prepared by dissolving 5 g of the transparent lumps or sticks in cold H<sub>2</sub>O and diluting to 100 cc), mix thoroly, dilute to volume, and filter. Transfer 80 cc of the filtrate, or as large an aliquot as possible, to a 100 cc flask; neutralize with a strong soln of NaOH (1+1), using phenolphthalein indicator; cool, dilute to the mark, and determine reducing sugars on an aliquot as directed under **XXXIV, 38**. Calculate to invert sugar.

Some dressings, particularly those containing starch, cannot be clarified by the above method. In such instances, remove the oil as directed under **XXIII, 8**, transfer the residue to a 250 cc flask with alcohol, 50% by volume, and proceed as directed under **XXVII, 28**.

46

**REDUCING SUGARS AFTER INVERSION—TENTATIVE**

Invert an aliquot of the soln, 45, as directed under **XXXIV, 23(b)**, and determine the reducing sugars in the inverted soln as directed under **XXXIV, 37**. Calculate to invert sugar.

47

**SUCROSE—TENTATIVE**

Subtract the percentage of invert sugar obtained before inversion, 45, from that obtained after inversion, 46, and multiply the difference by 0.95.

48

**TOTAL ACIDITY<sup>15</sup>—OFFICIAL**

Weigh about 15 g of the sample into a 500 cc Erlenmeyer flask, dilute to about 200 cc, and shake until all lumps of dressing are thoroly broken up. Titrate with 0.10 N NaOH, using neutral phenolphthalein, and calculate as acetic acid. In order to recognize the end point, have a duplicate sample at hand so that, by comparison, the first change of color may be noted.

49

**TOTAL NITROGEN<sup>16</sup>—OFFICIAL**

Weigh about 15 g of the sample into a 500 cc Kjeldahl flask and place on the steam bath until the egg is thoroly cooked and the oil separates readily. Cool and

add about 50 cc of petroleum ether; mix, and pour off the ether soln thru a small filter. Repeat the ether treatment twice, rinsing out as much oil as possible. Wash the filter with petroleum ether and add the filter paper to the sample in the flask. Determine N, using 35 cc of  $H_2SO_4$  for digestion, as directed in XXIII, 5.

50 TOTAL PHOSPHORIC ACID ( $P_2O_5$ )<sup>14</sup>—OFFICIAL

Use a 10 g sample and proceed as directed in XXIII, 16, except to use a Pt dish in place of the beaker and to burn off the oil before ashing in the muffle.

51 TOTAL FAT<sup>14</sup>—TENTATIVE

Use a 2 g sample and proceed as directed under XXIII, 8.

52 CALCULATION OF COMPOSITION<sup>14</sup>—TENTATIVE

When  $P$  = % total  $P_2O_5$  and  $N$  = % total nitrogen, then

% yolk =  $75.69 P - 1.802 N$ ;

% white =  $60.80 N - 114.59 P$ ;

% total egg = % yolk + % white;

% white in egg component =  $\frac{\% \text{ white}}{\% \text{ total egg}} \times 100$ ;

Vegetable oil = total fat - (yolk  $\times 0.3188$ );

Vinegar (4% acid strength) = total acidity as acetic  $\times 25$ ;

Minor constituents (sugar, salt, spices, stabilizers) = total solids - (yolk  $\times 0.5047$ ) - (white  $\times 0.1221$ ) - vegetable oil; and

Added water =  $100\% - \text{total egg} - \text{vegetable oil} - \text{vinegar} - \text{minor constituents}$ .

53 IDENTIFICATION OF OIL—TENTATIVE

Proceed as directed under XXXI, using the oil obtained by evaporating the petroleum ether extracts in the determination of reducing sugars, 45.

VINEGARS<sup>15</sup>

(Unless otherwise directed, express results as grams per 100 cc.)

54 PHYSICAL EXAMINATION—OFFICIAL

Note the appearance, color, odor, and taste.

55 PREPARATION OF SAMPLE—OFFICIAL

Mix thoroly and filter before proceeding with the analysis.

56 SPECIFIC GRAVITY—OFFICIAL

Determine the specific gravity at  $20/20^\circ$  by means of a pycnometer, as directed under XIV, 3.

57 SOLIDS—OFFICIAL

Measure 10 cc of the sample into a weighed, flat-bottomed Pt dish having a bottom diameter of 50 mm, evaporate on a boiling water bath for 30 min., and dry for exactly 2.5 hours in a water oven at the temp. of boiling  $H_2O$ . Cool in a desiccator and weigh. To obtain concordant results, it is necessary to use a dish of the size and shape stated and to dry for exactly the time specified.

58

## ASH—OFFICIAL

(a) Measure 25 cc of the vinegar into a weighed Pt dish, evaporate to dryness on a steam bath, and proceed as directed under XXVII, 8.

(b) Evaporate 25 cc of the sample to dryness as directed under (a) and heat in a muffle at low heat to expel inflammable gases. Treat the charred portion with a few cc of  $H_2O$ , and evaporate to dryness on a steam bath; replace in the muffle at low redness for 15 min. and continue the alternate evaporation and heating until a white or gray ash is obtained; cool in a desiccator and weigh.

Useful information may often be obtained by noting the odor given off by the solids during charring.

59

## SOLUBLE AND INSOLUBLE ASH—OFFICIAL

Treat the ash obtained, 58, as directed under XXXIV, 12.

60

## ALKALINITY OF THE SOLUBLE ASH—OFFICIAL

Proceed as directed under XXXIV, 13, using the soluble ash obtained under 59. Express the result as the number of cc of  $N$  acid required to neutralize the soluble ash from 100 cc of the vinegar.

In case the relation of the ash to the alkalinity of the soluble ash is abnormal, the composition of the ash should be studied, especially as to content of chlorides, sulfates, phosphates, and alkalies.<sup>16</sup>

61

## SOLUBLE PHOSPHORIC ACID—OFFICIAL

Proceed as directed under II, 9 or 12, using the soln obtained under 60. Express the result as mg of  $P_2O_5$  in 100 cc of vinegar.

62

## INSOLUBLE PHOSPHORIC ACID—OFFICIAL

Dissolve the water-insoluble ash obtained under 59 in approximately 50 cc of boiling  $HNO_3$  (1+8) and proceed as directed under II, 9 or 12. Express the result as mg of  $P_2O_5$  in 100 cc of vinegar.

63

## TOTAL ACIDS OFFICIAL

Dilute 10 cc of the sample with recently boiled and cooled  $H_2O$  until it appears slightly colored and titrate with 0.5  $N$  alkali, using phenolphthalein indicator. 1 cc of 0.5  $N$  alkali = 0.030 g of acetic acid.

64

## NON-VOLATILE ACIDS—OFFICIAL

Measure 10 cc of the vinegar into a 200 cc porcelain casserole, evaporate just to dryness, add 5–10 cc of  $H_2O$ , and again evaporate; repeat until at least 5 evaporations have been made. Add about 200 cc of recently boiled and cooled  $H_2O$  and titrate with 0.1  $N$  alkali soln, using phenolphthalein indicator. 1 cc of 0.1  $N$  alkali = 0.0060 g of acetic acid.

65

## VOLATILE ACIDS—OFFICIAL

To obtain the volatile acids subtract the quantity of non-volatile acids, 64, from the quantity of total acids, 63.

66

## TOTAL REDUCING SUBSTANCES BEFORE INVERSION OFFICIAL

Measure 25 cc of the sample into a 50 cc volumetric flask and add enough  $NaOH$  soln (1+1) nearly to neutralize the acid. Cool, dilute to the mark with  $H_2O$ , and

determine the reducing substances in 20 cc of the soln as directed under XXXIV, 37. If the quantity of reducing substances is very small, use 40 cc of the soln. Calculate the result as invert sugar (for malt vinegar as dextrose).

**67 TOTAL REDUCING SUBSTANCES AFTER INVERSION—OFFICIAL**

Invert 25 cc of the sample in a 50 cc volumetric flask with 5 cc of dilute HCl, as directed under XXXIV, 23(b) or (c). Neutralize with NaOH soln (1+1) and determine reducing substances as directed under 57.

**68 NON-VOLATILE REDUCING SUBSTANCES (SUGAR)—OFFICIAL**

(Useful in calculating the non-sugar solids.)

Evaporate 50 cc of the sample on a steam or water bath to a sirupy consistency, add 10 cc of H<sub>2</sub>O, and evaporate again. Repeat with 10 cc of H<sub>2</sub>O. Transfer the residue to a 100 cc volumetric flask with about 50 cc of warm H<sub>2</sub>O. Cool; invert with 10 cc of dilute HCl as directed under XXXIV, 23(b) or (c); nearly neutralize with NaOH soln (1+1); cool, dilute to the mark with H<sub>2</sub>O, and determine reducing substances in 20 cc or 40 cc, depending on the quantity present, as directed under XXXIV, 37. Calculate the result as invert sugar (for malt vinegar as dextrose). If the results for total reducing substances before and after inversion show the absence of sucrose, the inversion is unnecessary and may be omitted.

**69 VOLATILE REDUCING SUBSTANCES<sup>17</sup>—OFFICIAL**

When sucrose is absent, subtract the quantity of non-volatile reducing substances, 68, from the mean of the total reducing substances before inversion, 66, and after inversion, 67. When sucrose is present, subtract the quantity of non-volatile reducing substances, 68, from the quantity of total reducing substances after inversion, 67.

**70 ALCOHOL—OFFICIAL**

Measure 100 cc of the sample into a round-bottomed distillation flask. Make faintly alkaline with NaOH soln (1+1), distil almost 50 cc, dilute to 50 cc at the temp. of the sample, and determine the sp. gr. at 20°/20° by means of a pycnometer, XIV, 3. Obtain from Table 19, XLII, the percentage by volume noting that the alcoholic strength of the distillate is twice that of the original vinegar. Undue foaming may be obviated by adding a small piece of paraffin, free from volatile constituents.

**GLYCEROL<sup>18</sup>—OFFICIAL**

**71**

**REAGENTS**

(a) *Strong potassium dichromate soln.*—Dissolve 74.55 g of dry, recrystallized K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> in H<sub>2</sub>O; add 150 cc of H<sub>2</sub>SO<sub>4</sub>; cool, and dilute with H<sub>2</sub>O to 1 liter at 20°. 1 cc of this soln = 0.01 g of glycerol. Owing to the high coefficient of expansion of this strong soln it is necessary to make all volumetric measurements of the soln at the same temp. as that at which it was diluted to volume.

(b) *Dilute potassium dichromate soln.*—Measure 25 cc of the strong K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> soln at 20° into a 500 cc volumetric flask and dilute to the mark with H<sub>2</sub>O at room temp. 20 cc of this soln = 1 cc of (a).

(c) *Ferrous ammonium sulfate soln.*—Dissolve 30 g of crystallized ferrous ammonium sulfate in H<sub>2</sub>O, add 50 cc of H<sub>2</sub>SO<sub>4</sub>, cool, and dilute with H<sub>2</sub>O to 1 liter at room

temp. 1 cc of this soln = approximately 1 cc of (b). As its value changes slightly from day to day, it must be standardized against (b) whenever used.

(d) *Diphenylamine indicator*.—Dissolve 1 g of diphenylamine in 100 cc of  $\text{H}_2\text{SO}_4$ .

(e) *Retarder*.—Dilute 150 cc of phosphoric acid (sirupy) with 600 cc of  $\text{H}_2\text{O}$ , and add 250 cc of  $\text{H}_2\text{SO}_4$ .

(f) *Milk of lime*.—Introduce 150 g of  $\text{CaO}$ , selected from clean hard lumps, prepared preferably from marble, into a large porcelain or iron dish; slake with  $\text{H}_2\text{O}$ , cool, and add sufficient  $\text{H}_2\text{O}$  to make 1 liter.

(g) *Silver carbonate*.—Dissolve 0.1 g of  $\text{Ag}_2\text{SO}_4$  in about 50 cc of  $\text{H}_2\text{O}$ , add an excess of  $\text{Na}_2\text{CO}_3$  soln, allow the precipitate to settle, and wash with  $\text{H}_2\text{O}$  several times by decantation until the washings are practically neutral. This reagent must be freshly prepared immediately before use.

## 72

## DETERMINATION

Make evaporations on a water bath maintained at a temp. of 85–90°. The area of the dish exposed to the bath should not be greater in circumference than that covered by the liquid inside.

Evaporate 100 cc of the vinegar to 5 cc, add 20 cc of  $\text{H}_2\text{O}$ , and again evaporate to 5 cc to expel acetic acid. Treat the residue with about 5 g of 40-mesh sand and 15 cc of the milk of lime and evaporate almost to dryness, with frequent stirring, avoiding the formation of a dry crust or evaporation to complete dryness. Treat the moist residue with 5 cc of  $\text{H}_2\text{O}$ ; rub into a homogeneous paste; add slowly 45 cc of absolute alcohol, washing down the sides of the dish to remove adhering paste; and stir thoroughly. Heat the mixture on a water bath, with constant stirring, to incipient boiling; transfer to a suitable vessel; and centrifuge. Decant the clear liquid into a porcelain dish and wash the residue with several small portions of hot alcohol, 90% by volume, by aid of the centrifuge. (If a centrifuge is not available, decant the liquid thru a folded filter into a porcelain dish. Wash the residue repeatedly with small portions of hot 90% alcohol, twice by decantation, and then by transferring all the material to the filter. Continue the washing until the filtrate amounts to 150 cc.) Evaporate to a sirupy consistency, add 10 cc of absolute alcohol to dissolve this residue, and transfer to a 50 cc glass-stoppered cylinder, washing the dish with successive small portions of absolute alcohol until the volume of the soln is 20 cc. Add 3 portions of 10 cc each of anhydrous ether, shaking thoroly after each addition. Let stand until clear, pour off thru a filter, and wash the cylinder and filter with a mixture of 2 volumes of absolute alcohol and 3 of anhydrous ether. If a heavy precipitate has formed in the cylinder, centrifuge at low speed, decant the clear liquid, and wash 3 times with 20 cc portions of the alcohol-ether mixture, shaking the mixture thoroly each time and separating the precipitate by means of the centrifuge. Wash the paper with the alcohol-ether mixture and evaporate the filtrate and washings on the water bath to about 5 cc, add 20 cc of  $\text{H}_2\text{O}$ , and again evaporate to 5 cc; again add 20 cc of  $\text{H}_2\text{O}$  and evaporate to 5 cc; finally add 10 cc of  $\text{H}_2\text{O}$  and evaporate to 5 cc.

These evaporations are necessary to remove all the ether and alcohol, and when conducted at 85–90° they result in no loss of glycerol if the concentration of the latter is less than 50%.

Transfer the residue with hot  $\text{H}_2\text{O}$  to a 50 cc volumetric flask, cool, add the  $\text{Ag}_2\text{CO}_3$  prepared from 0.1 g of  $\text{Ag}_2\text{SO}_4$ , shake, and allow to stand 10 min. Then add 0.5 cc of basic Pb acetate soln, XXXIV, 18(a); shake occasionally, and allow to stand 10 min. Make up to the mark, shake well, filter, rejecting the first portion of the filtrate, and pipet 25 cc of the clear filtrate into a 250 cc volumetric flask.

Add 1 cc of  $H_2SO_4$  to precipitate the excess of Pb and then 30 cc of Reagent (a). Add carefully 24 cc of  $H_2SO_4$ , rotating the flask gently to mix the contents and avoid violent ebullition, and then place in a *boiling* water bath for exactly 20 min. Remove the flask from the bath, dilute, cool, and make up to the mark at room temp. The quantity of strong dichromate soln used must be sufficient to leave an excess of about 12.5 cc at the end of the oxidation, the quantity given above (30 cc) being sufficient for ordinary vinegar containing about 0.35 g or less of glycerol per 100 cc.

Standardize the ferrous ammonium sulfate soln by pipetting 20 cc into a 250 cc beaker, adding 20 cc of the retarder, 4 drops of the indicator, and about 100 cc of  $H_2O$ . Titrate with the dilute potassium dichromate soln until the liquid assumes a dark green color, then add the dichromate slowly dropwise, stirring continuously, until the color changes from a blue gray to a deep violet. Designate the cc of dilute dichromate soln used as (a). In place of the dilute dichromate soln, substitute a buret containing the oxidized glycerol with an excess of the strong dichromate soln and titrate 20 cc of the ferrous ammonium sulfate soln as before, designating the cc used as (b).

From the figures obtained calculate the glycerol (g per 100 cc of vinegar) by the following formula:

$$G = \left( D - \frac{250(a)}{20(b)} \right) 0.02, \text{ in which}$$

$G$  = g of glycerol per 100 cc of vinegar, and  $D$  = cc of the strong potassium dichromate soln used to oxidize the glycerol.

## 73

## COLOR—OFFICIAL

Determine the depth of color in a Lovibond tintometer by good reflected daylight, using  $\frac{1}{2}$ - or 1-inch cell and the brewer's scale. Report the result in terms of  $\frac{1}{2}$ -inch cell and so state.

## 74

COLOR REMOVED BY FULLERS' EARTH<sup>14</sup>—TENTATIVE

To 50 cc of the sample add 10 g of fullers' earth, shake at intervals for 30 min., and filter thru a folded filter. Place as much of the filtrate as is available into a colorimeter tube and place an equal volume of the original sample in a corresponding tube; dilute both with  $H_2O$  to a volume of 50 cc and compare colors. Express the result as percentage of color removed. Not all fullers' earth is satisfactory for this test. The efficiency of the reagent should be determined on a sample of distilled vinegar known to be colored with caramel.

## 75

## POLARIZATION\*—TENTATIVE

Whenever possible, polarize in a 200 mm tube without decolorizing. Report results on basis of 200 mm tube in degrees Ventzke. When necessary, decolorize as follows:

(a) To 50 cc of the sample add a measured quantity of saturated neutral Pb acetate soln, avoiding an excess of Pb; filter, remove Pb with powdered anhydrous K oxalate, and filter. Polarize and correct for the dilution with Pb acetate soln.

(b) To 50 cc of the sample add decolorizing C, avoiding an excessive amount or length of treatment. Filter thru a double paper and polarize.

## 76

## SULFATES—OFFICIAL

To 100 cc of the absolutely clear sample, add 2 cc of approximately normal HCl; heat to boiling; add 10 cc of hot  $BaCl_2$  soln (1 g per 100 cc), dropwise, and continue the boiling 5 min., keeping the volume approximately constant by adding hot  $H_2O$



from time to time as required. Allow to stand until the supernatant liquid is clear. Overnight is convenient, but this time should not be exceeded. Filter on an ashless filter paper or a weighed Munroe crucible.<sup>22</sup> Wash free from chlorides with hot  $\text{H}_2\text{O}$ , dry, ignite at a low red heat, cool, and weigh. Express the result as mg of  $\text{SO}_3$  in 100 cc of vinegar.

77

FORMIC ACID<sup>23</sup>—OFFICIAL

Use the apparatus described under XXXII, 40. Introduce 100 cc of the sample into flask (A); add 0.4–0.5 g of tartaric acid and place in position as shown in the figure, having previously charged the flask B with a suspension of 15 g of  $\text{CaCO}_3$  in 100 cc of  $\text{H}_2\text{O}$ . Heat the contents of flasks A and B to boiling and distil with steam from the generator S, the vapor passing first thru the sample in flask A, then thru the boiling suspension of  $\text{CaCO}_3$  in flask B, after which it is condensed and measured in the receiver C. Maintain the volume of liquid in flask B as nearly constant as possible and reduce the volume of the sample in flask A to 30–40 cc by heating with small Bunsen flames. Continue the distillation until 1 liter of distillate is collected. Disconnect the apparatus, filter the  $\text{CaCO}_3$  suspension, and wash the  $\text{CaCO}_3$  that remains on the paper with a little hot  $\text{H}_2\text{O}$ . Render the filtrate faintly acid with  $\text{HCl}$ , add 10–15 cc of  $\text{HgCl}_2$  reagent, XXXII, 39(b), mix, and heat on a boiling water bath for 2 hours. Filter on a weighed Gooch crucible and wash the precipitate thoroughly with cold  $\text{H}_2\text{O}$  and finally with a little alcohol. Dry in a boiling water oven for 30 min., cool in a desiccator, weigh, and calculate the weight of  $\text{HCOOH}$  present by multiplying the weight of the precipitate by 0.0975.

## TARTARIC ACID AND TARTRATES

78

*Qualitative Test—Official*

Evaporate 50 cc of the vinegar in a porcelain dish to a volume of about 10 cc, filter into a test tube, add 1 cc of 25%  $\text{CaCl}_2$  soln and 2 cc of 50%  $\text{NH}_4$  acetate soln, and allow to stand overnight. In the presence of tartaric acid a deposit of Ca tartrate is formed, the crystals of which may be identified under the microscope by their characteristic form.

79

## TOTAL TARTARIC ACID—OFFICIAL

Evaporate 200 cc of the sample to a sirupy consistency to remove excess of acetic acid, dilute to the original volume with  $\text{H}_2\text{O}$  in a volumetric flask, determine the acidity as directed under 63, and determine total tartaric acid in a 100 cc aliquot as directed under XV, 26, using 20 cc of alcohol in the precipitation instead of 15 cc.

## FREE MINERAL ACIDS

80

*I. Logwood Method<sup>24</sup>—Tentative*

Prepare an extract of logwood as follows: Pour 100 cc of boiling  $\text{H}_2\text{O}$  upon 2 g of fresh logwood chips, allow the infusion to stand for a few hours, and filter. Place several drops of the liquid on a porcelain surface and dry on a water bath. Add to one of the spots a drop of the vinegar to be tested (after concentration if desirable) and evaporate to dryness. A yellow tint remains if free mineral acids are absent, a red tint if they are present.

81

*II. Methyl Violet Method—Tentative*

Add 5–10 cc of  $\text{H}_2\text{O}$  to 5 cc of vinegar, and after mixing well add 4 or 5 drops of methyl violet soln (1 part of methyl violet 2B in 10,000 parts of  $\text{H}_2\text{O}$ ). A blue or green coloration indicates the presence of a free mineral acid.

82

*III. Quantitative Method—Tentative*

To a measured quantity of the sample add a measured excess of standard alkali, evaporate to dryness, incinerate, and titrate the ash with standard acid, using methyl orange indicator. The difference between the number of cc of alkali first added and the number of cc of acid needed to titrate the ash represents the free mineral acid present.

83

**METALS—TENTATIVE**

Proceed as directed under **XXIX**.

84

**DEXTRIN (QUALITATIVE TEST)—TENTATIVE**

Evaporate 100 cc of the vinegar to a volume of about 15 cc. Add slowly and with constant stirring 200 cc of 95% alcohol and allow to stand overnight. The precipitate formed should be tested for dextrin by the optical rotation and color reaction with I.

85

**SPICES AND ADDED PUNGENT MATERIALS (QUALITATIVE TEST)—TENTATIVE**

Neutralize exactly a portion of the vinegar and test by taste and smell. Agitate the liquid with ether in a separatory funnel, remove, evaporate the ethereal layer, and note the odor and taste of the residue.

86

**COLORING MATTERS—TENTATIVE**

Proceed as directed under **XXI**.

87

**PRESERVATIVES—OFFICIAL**

Proceed as directed under **XXXII**.

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## XXXIV. SUGARS AND SUGAR PRODUCTS

### SUGARS, SIRUPS, AND MOLASSES

1

#### PREPARATION OF SAMPLE—OFFICIAL

(a) *Solids (sugars, etc.)*.—Grind, if necessary, and mix thoroly to secure uniform samples. In the case of raw sugars mix thoroly and in the shortest possible time on a glass plate with a spatula, reducing lumps when present with a glass or iron rolling pin; or mix thoroly and in the shortest possible time in a large, clean, dry mortar, using a pestle to reduce lumps when present.

(b) *Semi-solids (massecuites, etc.)*.—Weigh 50 g of the sample, dissolve crystals of sugar in the minimum amount of  $H_2O$ , wash into a 250 cc volumetric flask, fill to the mark, and mix thoroly; or weigh 50 g of the sample and dilute with  $H_2O$  to 100 g. If insoluble material remains, mix uniformly by shaking before taking aliquots or weighed portions for the various determinations.

(c) *Liquids (molasses, sirups, etc.)*.—Mix materials thoroly. If crystals of sugar are present, dissolve them either by heating gently (avoiding loss of  $H_2O$  by evaporation) or by weighing the whole mass, then adding  $H_2O$ , heating until completely dissolved and after cooling, reweighing. Calculate all results to the weight of the original substance.

2

#### MOISTURE—OFFICIAL

##### *Direct Drying*

Dry 2–5 g of the prepared sample, 1(a), in a flat dish (Ni, Pt, or Al) at the temp. of boiling  $H_2O$  for 10 hours; cool in a desiccator and weigh. Dry again for an hour or until the change in weight is not more than 2 mg. In the case of sugars of large grain, heat at  $105$ – $110^\circ$  to expel the last traces of occluded  $H_2O$ .<sup>1</sup> Report the loss in weight as moisture.

3

##### *Drying upon Pumice Stone—Official*

(Applicable to massecuites, molasses and other liquid and semiliquid products.)

Prepare pumice stone of 2 grades of fineness, one of which will pass thru a 1 mm sieve, the other thru a 6 mm but not a 1 mm sieve. Digest each with  $H_2SO_4$  (1+4) for 8 hours on a steam bath. Wash free from acid and heat to dull redness. Make the determination in a flat metallic dish 60 mm in diameter. Place a layer of the fine pumice stone, 3 mm in thickness, on the bottom of the dish, then a layer of the coarse pumice stone, 6–10 mm in thickness; dry, and weigh. Dilute the sample with a weighed portion of  $H_2O$  so that the diluted material shall contain 20–30% of solid matter. Weigh into the dish, prepared as described, the quantity of diluted sample to yield, approximately, 1 g of dry matter. If this weighing can not be made rapidly, use a weighing bottle provided with a cork thru which a pipet passes. Dry at  $70^\circ$  under a pressure of not to exceed 100 mm of Hg, making trial weighings at intervals of 2 hours toward the end of the drying period until the change in weight does not exceed 2 mg. Report the loss in weight as moisture. For substances containing little or no levulose or other readily decomposable substance, the drying may be made in a water oven at the temp. of boiling  $H_2O$ .

4

*Drying upon Quartz Sand—Official*

(Applicable to massecuites, molasses and other liquid and semiliquid products.)

Digest pure quartz sand that will pass a 40-mesh but not a 60-mesh sieve with HCl, wash free from acid, dry, and ignite. Preserve in a stoppered bottle. Place 25–30 g of the prepared sand and a short stirring rod in a dish approximately 55 mm in diameter and 40 mm in depth, fitted with a cover. Dry thoroly, cover dish, cool in a desiccator, and weigh immediately. Then add sufficient diluted sample of known weight to yield approximately 1 g of dry matter and mix thoroly with the sand. Heat on a steam bath for 15–20 min., stirring at intervals of 2–3 min., or until the mass becomes too stiff to manipulate readily. Dry at 70° under a pressure of not to exceed 100 mm of Hg, making trial weighings at 2 hour intervals toward the end of the drying period (about 18 hours) until the change in weight does not exceed 2 mg.

For materials containing no levulose or other readily decomposable substance the material may be dried at atmospheric pressure in a water oven at the temp. of boiling H<sub>2</sub>O, heated for 8–10 hours, cooled in a desiccator, and weighed, the heating and weighing being repeated until the loss in 1 hour does not exceed 2 mg. Report the loss in weight as moisture.

(Dry sand, as well as the dried sample, will absorb an appreciable quantity of moisture on standing over most desiccating agents, so all weighings should be made as quickly as possible after cooling in the desiccator.)

## SOLIDS

(Not accurate when applied to low-grade sugar products, molasses, and other materials containing large quantities of non-sugar solids, but extensively used for approximate results.)

5

*By Means of a Spindle—Official*

The density of juices, sirups, etc., is conveniently determined by means of the Brix or Baumé hydrometer, preferably the former, as the graduations of the scale give close approximations to the percentages of total solids. A table for comparison of degrees Brix (a density scale indicating directly the percentage by weight of pure sucrose in pure solns), degrees Baumé (modulus 145), sp. gr. at 20/4°, and sp. gr. at 20/20° is given under XLII, 3.

Use a spindle graduated in tenths and as limited as possible in the range of degrees recorded and a cylinder of sufficient diameter to permit the spindle to come to rest without touching the sides. Allow the soln to come as nearly as practicable to the same temp. as the air at the time of reading, and if this varies more than 1° from the temp. at which the spindle was graduated, 20°, apply a correction according to XLII, 4. Before taking the density of a juice, allow it to stand in the cylinder until all air bubbles have escaped and all fatty or waxy matters have come to the top and been skimmed off. (Air bubbles may be conveniently removed, especially in the case of solns of high density, by applying vacuum to the cylinder by means of a tube passing thru a stopper inserted in the top of the cylinder.) If the sample is too dense to determine the density directly, dilute a weighed portion with a weighed quantity of H<sub>2</sub>O, or dissolve a weighed portion and dilute to a known volume with H<sub>2</sub>O. In the first instance the percentage of total solids is calculated by the following formula: Percentage of solids in the undiluted material =  $\frac{WS}{w}$ , in which

$S$  = percentage of solids in the diluted material,

$W$  = weight of the diluted material; and

$w$  = weight of sample taken for dilution.

When the dilution is made to a definite volume, use the following formula:

Percentage of solids in the undiluted material =  $\frac{VDS}{w}$ , in which

$V$  = volume of the diluted soln at a given temp.;

$D$  = sp. gr. of the diluted soln at the same temp.;

$S$  = percentage of solids in the diluted soln at the same temp.; and

$w$  = weight of the sample taken for dilution.

*By Means of a Pycnometer<sup>3</sup>—Official*

(a) *Specific gravity (in vacuo or in air).*—Determine the sp. gr. of the soln at 20/4°, 20/20° in vacuo or 20/20° in air as directed under XIV, 3, and ascertain the corresponding percentage by weight of sugar solids from the appropriate table, LXII, 3, or 5. When the density of the substance is too high for a direct determination, dilute and then calculate the sucrose content of the original material as directed under 5.

(b) *Specific gravity of molasses.*—Use a special 100 cc volumetric flask with a neck of approximately 8 mm inside diameter. Weigh the flask empty and then fill it with molasses, using a long-stemmed funnel reaching below the graduation mark, until the level of the molasses is up to the lower end of the neck of the flask. (The flow of molasses may be stopped by inserting a glass rod of suitable size into the funnel so as to close the stem opening.) Remove the funnel carefully to prevent the molasses coming in contact with the neck, and weigh flask and molasses. Add H<sub>2</sub>O almost up to the graduation mark, running it down the side of the neck to prevent mixing with the molasses. Allow to stand several hours or overnight to permit the escape of bubbles. Place the flask in a constant temp. H<sub>2</sub>O bath, preferably at 20°, and leave until it reaches the temp. of the bath; then make to volume at that temp. with H<sub>2</sub>O. Weigh. Reduce the weight of the molasses to vacuo and calculate the density. Ascertain the corresponding Brix or Baumé reading from XLII, Table 3.

*Example:*

	grams
$A$ , H <sub>2</sub> O capacity of flask 20°	= 99.823
$B$ , weight of molasses 20°	= 132.834
$C$ , weight of molasses and H <sub>2</sub> O 20°	= 137.968
$C - B$ = weight of water added	= 5.134
$A - (C - B)$ = weight of H <sub>2</sub> O occupying space of molasses	= 94.689
$\frac{132.834}{94.689}$	= 1.403 sp. gr. molasses.

7

*By Means of a Refractometer<sup>4</sup>—Official*

(Applicable only to liquid samples containing no undissolved solids.)

Determine the refractometer reading of the soln at 20° and obtain the corresponding percentage of dry substance from either the direct reading, if a sugar refractometer is used, or from Table 6, XLII, if the instrument gives readings in terms of refractive index. Circulate H<sub>2</sub>O at a constant temp., preferably 20°, thru the jackets of the refractometer or thru the trough of the immersion instrument, long enough to allow the temp. of the prisms and of the sample to reach an equilibrium, con-

tinuing the circulation during observations and taking care that constant temp. is maintained. If the determination is made at a temp. other than 20°, or if the humidity causes condensation of moisture on the exposed faces of the prisms, make the measurements at room temp. and correct the readings to the standard temp. of 20° according to Table 7, XLII. If the soln is too dark to be read in the instrument, dilute with a concentrated sugar soln; never use H<sub>2</sub>O for this purpose. Mix weighed quantities of the soln under examination and a soln of pure sugar of about the same strength, and obtain the quantity of dry substance in the former by the following formula:

$$x = \frac{(A+B)C - BD}{A}, \text{ in which}$$

$x$  = percentage of dry substance to be found;

$A$  = weight in g of the sample mixed with  $B$ ;

$B$  = weight in g of pure sugar soln used in the dilution;

$C$  = percentage of dry substance in the mixture  $A + B$  obtained from the refractive index; and

$D$  = percentage of dry substance in the pure sugar soln obtained from its refractive index.

#### ASH—OFFICIAL

##### *Carbonated Ash*

8

##### *Method I.*

Heat 5–10 g of the sample in a 50–100 cc Pt dish at 100° until the H<sub>2</sub>O is expelled, add a few drops of pure olive oil, and heat slowly over a flame until swelling ceases. Then place the dish in a muffle and heat at low redness until a white ash is obtained. Treat the residue with a little (NH<sub>4</sub>)<sub>2</sub>CO<sub>3</sub> soln, re-evaporate, and heat again in the muffle at a very dull red heat to constant weight.

9

##### *Method II.*

Carbonize 5–10 g of the sample in a 50–100 cc Pt dish at a low heat and treat the charred mass with hot H<sub>2</sub>O to dissolve the soluble salts. (In the case of low-purity products the addition of a few drops of pure olive oil, as in 8, may be desirable.) Filter thru an ashless filter, ignite filter and residue to a white ash, add the filtrate of soluble salts, evaporate to dryness, and ignite gently. Treat the residue with a little (NH<sub>4</sub>)<sub>2</sub>CO<sub>3</sub> soln, re-evaporate, and heat again in the muffle at a very dull red heat to constant weight.

10

##### *Sulfated Ash*

Weigh 5 g of the sample into a 50–100 cc Pt dish, add 0.5 cc of H<sub>2</sub>SO<sub>4</sub>, ignite gently until the sample is well carbonized, and then burn in a muffle at low redness. Cool, add a few drops more of H<sub>2</sub>SO<sub>4</sub>, heat until fully volatilized, cool, and weigh. Express the result as percentage of sulfated ash.

11

#### MINERAL CONSTITUENTS—OFFICIAL

Proceed as directed under XII.

12

#### SOLUBLE AND INSOLUBLE ASH—OFFICIAL

Ash the material as directed under 8 or 9. Add H<sub>2</sub>O to the ash in the Pt dish, heat nearly to boiling, filter thru an ashless filter, and wash with hot H<sub>2</sub>O until the com-

bined filtrate and washings measure about 60 cc. Return the filter and contents to the Pt dish, ignite carefully, cool, and weigh. Calculate the percentages of water-soluble and water-insoluble ash.

## 13

## ALKALINITY OF SOLUBLE ASH—OFFICIAL

Cool the filtrate from 12 and titrate with 0.1 *N* HCl, II, 19(a), using methyl orange indicator, VI, 3(f). Express the alkalinity in terms of the number of cc of normal acid per 100 g of sample.

## 14

## ALKALINITY OF INSOLUBLE ASH—OFFICIAL

Add an excess of 0.1 *N* HCl (usually 10–15 cc) to the ignited insoluble ash in the Pt dish, under 12, heat to incipient boiling on an asbestos plate, cool, and titrate the excess of HCl with 0.1 *N* NaOH, using methyl orange indicator. Express the alkalinity in terms of the number of cc of normal acid per 100 g of the sample.

## 15

## MINERAL ADULTERANTS IN THE ASH—TENTATIVE

In a large porcelain evaporating dish, mix 100 g of the sample with about 35 g of  $\text{H}_2\text{SO}_4$  and evaporate to a sirupy consistency. Pass an electric current thru it while stirring by placing one Pt electrode in the bottom of the dish near one side and attaching the other to the lower end of the glass rod with which the contents are stirred. Begin with a current of about 1 ampere and gradually increase to 4. In 10–15 min. the mass is reduced to a fine dry char, which may be readily burned to a white ash in the original dish over a free flame or in a muffle.

This method is preferred to the ordinary method of heating with  $\text{H}_2\text{SO}_4$ , especially in the case of molasses, because if properly manipulated the material comes quickly into the form of a very finely divided char or powder that is especially adapted for subsequent quick ignition.

If an electric current is not available, treat in a large porcelain dish 100 g of the saccharine soln, evaporated to a sirupy consistency, with sufficient  $\text{H}_2\text{SO}_4$  to carbonize the mass thoroly and ignite in the usual manner.

The following adulterants may be present: salts of tin, used in molasses to bleach; mineral pigments, such as  $\text{PbCrO}_4$ , in yellow confectionery; oxide of iron, sometimes used to simulate the color of chocolate, and Cu. These elements may be detected by the usual qualitative tests.

## 16

## NITROGEN—OFFICIAL

Determine N in 5 g of the material as directed under II, 21, 23, or 25, using a larger quantity of the  $\text{H}_2\text{SO}_4$  if necessary for complete digestion.

## SUCROSE—POLARIMETRIC METHODS

## GENERAL PROCEDURE

## 17

(a) *Directions for Raw Sugars—Official*

(Rules of the International Commission for Unifying Methods of Sugar Analysis<sup>6</sup>.)

"In general all polarizations are to be made at 20°.

"The verification of the saccharimeter must also be made at 20°. For instruments using the Ventzke scale 26 g of pure dry sucrose, weighed in air with brass weights, dissolved in  $\text{H}_2\text{O}$  so that 100 cc of soln is obtained at 20° and polarized in a room or cabinet, the temp. of which is also 20°, must give a saccharimeter reading of exactly 100.00. The temp. of the sugar soln during polarization must be kept constant at 20°."

According to the determination of Bates and Jackson<sup>7</sup> at the U. S. Bureau of Standards, the Ventzke scale saccharimeter reading for 26 g of pure dry sucrose under the above conditions is 99.895. The Ventzke scale reading has been re-determined by Balch and Hill<sup>8</sup> and by Zerban, Gamble and Hardin,<sup>9</sup> who found values of 99.907° and 99.912°, respectively. The average value of these three independent observers is 99.90.

"For countries where the mean temp. is higher than 20°, saccharimeters may be adjusted at 30° or any other suitable temp., under the conditions specified above, provided the sugar soln be diluted to final volume and polarized at this same temp.

"In determining the polarization of substances containing sugar employ only half-shade instruments."

The saccharimeter used may have either a single or double wedge and should be a half-shadow instrument with either double or triple field.

"During the observation keep the apparatus in a fixed position and so far removed from the source of light that the polarizing Nicol is not warmed.

"As sources of light, employ lamps which give a strong illumination, such as triple gas burner with metallic cylinder, lens, and reflector; gas lamps with Auer (Welsbach) burner; electric lamp; petroleum duplex lamp; or Na light. Whenever there is any irregularity in the sources of light such as that due to the convolutions of the filament in the case of electric light or to the meshes of the gauze in the case of the Welsbach light, place a thin ground-glass plate between the source of light and the polariscope so as to render the illumination uniform.

"Before and after each set of observations the chemist must satisfy himself of the correct adjustment of his saccharimeter by means of standardized quartz plates. He must also previously satisfy himself of the accuracy of his weights, polarization flasks, observation tubes, and cover-glasses. (Scratched cover-glasses must not be used.) Make several readings and take the mean thereof, but no one reading may be neglected."

The quartz plates are standardized to read to the second decimal place, and by their use a quick and at the same time accurate test can be made. In using such plates for testing saccharimeters, it is necessary that the instrument, as well as the plate, be at 20° before a reading is made. Different points of the scale, preferably 20°, 50°, 80°, and 100° (sugar scale), should be tested against the plates. The scale may also be standardized by means of a carefully calibrated telescopic control tube.<sup>9</sup> A new type of telescopic control tube of high accuracy has recently been described by Zerban, Gamble and Hardin.<sup>8</sup>

"In determining the polarization use the whole normal weight for 100 cc or a multiple thereof for any corresponding volume.

"As clarifying and decolorizing agents use basic acetate of lead . . . , alumina cream, or concentrated soln of alum. Boneblack and decolorizing powders are to be excluded."

Whenever reducing sugars are determined in the soln for polarizing, use only neutral Pb acetate for clarification, as basic Pb acetate causes precipitation of some of the reducing sugars. In addition to the clarifying agents mentioned, basic Pb nitrate has been made official by the Association.

"After bringing the soln exactly to the mark at the proper temp., and after wiping out the neck of the flask with filter paper, pour all the well-shaken clarified sugar soln on a rapidly acting, dry filter. Reject the first portion of the filtrate and use the remainder, which must be perfectly clear, for polarization."

Cover the funnel at the start of filtration. It is advisable to reject the first 25 cc that runs thru, and use the remainder for polarization. In no case should the whole



soln or any part be returned to the filter. If cloudy after the 25 cc has been rejected, begin a new determination.

"Whenever white light is used in polarimetric determinations, the same must be filtered thru a soln of  $K_2Cr_2O_7$  of such a concentration that the percentage content of the soln multiplied by the length of the column of the soln in centimeters is equal to nine."

This concentration must be doubled in polarizing carbohydrate materials of high rotation dispersion, such as commercial glucose, etc.

(b) *Conversion Factors of Different Saccharimeter Scales*

1 Ventzke Sugar Scale	= 0.34657° Angular Rotation D
1° Angular Rotation D*	= 2.88542° Ventzke Sugar Scale
Normal weight Ventzke Scale	= 26.026 grams
1° Bureau Standards Scale	= 0.34620° Angular Rotation D
1° Angular Rotation D	= 2.88850° Bureau Standards Scale
Normal weight Bur. Stand. Scale	= 26.000 grams
1° Bidecimal Scale	= 0.26622° Angular Rotation D
1° Angular Rotation D	= 3.75629° Bidecimal Scale
Normal weight Bidecimal Scale	= 20.000 grams
1° French Sugar Scale	= 0.21666° Angular Rotation D
1° Angular Rotation D	= 4.61553° French Sugar Scale
Normal weight French Scale	= 16.29 grams
1° Wild Sugar Scale	= 0.13284° Angular Rotation D
1° Angular Rotation D	= 7.52814° Wild Sugar Scale
Normal weight Wild Sugar Scale	= 10.00 grams

18 *Preparation and Use of Clarifying Reagents*<sup>10</sup>—Official

(a) *Basic lead acetate soln.*—Boil 430 g of neutral Pb acetate, 130 g of litharge, and 1 liter of  $H_2O$  for 30 min. Allow the mixture to cool and settle and then dilute the supernatant liquid to a sp. gr. of 1.25 with recently boiled  $H_2O$ . Solid basic Pb acetate may be substituted for the normal salt and litharge in the preparation of the soln. (This reagent is used primarily for clarifying dark colored cane, sorghum, and beet products when sucrose is determined by polariscopic methods.)

(b) *Alumina cream.*—Prepare a cold saturated soln of alum in  $H_2O$ . Add  $NH_4OH$  with constant stirring until the soln is alkaline to litmus, allow the precipitate to settle, and wash by decantation with  $H_2O$  until the wash  $H_2O$  gives only a slight test for sulfates with  $BaCl_2$  soln. Pour off the excess of  $H_2O$  and store the residual cream in a stoppered bottle. (Alumina cream is suitable for clarifying light colored sugar products or as an adjunct to other agents when sugars are determined by polariscopic or reducing sugar methods.)

(c) *Dry basic lead acetate.*—Obtained as a dry powdered salt and should contain 72.8% of Pb, which corresponds to a composition of  $3 Pb(C_2H_3O_2)_2 \cdot 2 PbO$ . Of this salt, about  $\frac{1}{2}$  g = 1 cc of the basic Pb acetate soln described under (a). In making the clarification, add a small quantity of the dry salt to the sugar soln after completion to volume and shake; then add more salt and shake again, repeating the addition

\* The designation D refers to sodium light of 5893 Ångström.

until precipitation is complete, but avoiding any excess. When molasses or any other substance producing a heavy precipitate is being clarified, add some dry, coarse sand to break up the pellets of basic Pb acetate and precipitate. (Dry basic Pb acetate can also be used in place of a soln of this salt in clarifying cane, sorghum, and beet products.)

(d) *Neutral lead acetate*.—Prepare a saturated soln of neutral Pb acetate and add it to the sugar soln before completing to volume. (This reagent may be used for clarifying light-colored sugar products when sugars are determined by polariscopic methods, and its use is imperative when reducing sugars are determined in the soln used for polarization.)

(e) *Basic lead nitrate*.—(1) Dissolve 250 g of  $\text{Pb}(\text{NO}_3)_2$  in  $\text{H}_2\text{O}$  and make up to 500 cc. (2) Dissolve 25 g of  $\text{NaOH}$  in  $\text{H}_2\text{O}$  and make up to 500 cc. In making the clarification, add equal quantities of (1) and (2) to the sugar soln, shake, and add more if complete precipitation has not occurred, but avoid an excess. Then complete the volume with  $\text{H}_2\text{O}$ . (This reagent is used for the same purposes as the one described under 18(a).) When this soln is used for clarification, the factor in the Clerget determination by acid becomes 143.5.

#### 19 *Temperature Corrections for the Polarization of Sugars*<sup>11</sup>—Tentative

(a) *Refined sugars*.—The polarizations of sugars testing 99 or above, when made at a temp. other than  $20^\circ$ , may be calculated to polarizations at  $20^\circ$  by the following formula:

$$P_{20} = p^t(1 + 0.0003(t - 20)), \text{ in which}$$

$p^t$  = the polarization at the temp. read; and  
 $t$  = the temp. at which the polarization is read.

(b) *Raw cane sugars*.—The polarizations of raw cane sugars, when made at temps. other than  $20^\circ$ , may be calculated to polarizations at  $20^\circ$  by the following formula:

$$P_{20} = p^t + 0.0015(p^t - 80)(t - 20), \text{ in which}$$

$p^t$  and  $t$  are the same as in the formula under (a).

When the percentage of levulose in the sugar is known (which in the case of honeys and sugar cane products is approximately  $\frac{1}{4}$  the reducing sugars), the following formula should be used:

$$P_{20} = p^t + 0.0003S(t - 20) - 0.00812L(t - 20), \text{ in which}$$

$p^t$  and  $t$  are the same as in the formula under (a);  
 $S$  = the percentage of sucrose; and  
 $L$  = the percentage of levulose.

These formulas give results agreeing closely with the polarizations obtained at  $20^\circ$  if the sugar is of average normal composition.

#### 20

#### *Mutarotation*

Products, such as honey and commercial glucose, that contain dextrose or other reducing sugars in crystalline form or in soln at high density may exhibit the phenomenon of mutarotation under the conditions prevailing during analysis. Only constant rotation should be used in polarimetric methods. To obtain this, allow the soln prepared for polarization to stand overnight before making the reading. If it is desired to make the reading immediately, heat the neutral (pH approximately 7.0) soln to boiling for a few moments or add a few drops of  $\text{NH}_4\text{OH}$  before completing to volume; or, if the soln has been made to volume, add dry  $\text{Na}_2\text{CO}_3$  until just distinctly alkaline to litmus paper. (Do not allow the slightly alkaline solns to

stand at such high temps. or for such lengths of time as to cause destruction of fructose.) Determine the completion of mutarotation by making readings at 15–30 min. intervals until these become constant.

#### SUCROSE IN THE ABSENCE OF RAFFINOSE

##### *1. By Polarization Before and After Inversion with Invertase<sup>12</sup>—Official*

###### 21

###### REAGENT

*Invertase soln.*—Commercial invertase preparations are available on the market. If it is desired to prepare the soln in the laboratory, the procedure described under (1) may be used. In either case the preparation may be further purified and concentrated by the ultrafiltration method described under (3). Commercial preparations may also be purified by dialysis and then reconcentrated by evaporating in vacuo at a temp. not to exceed 40°.

(1) *Crude invertase soln.*—Mix yeast with H<sub>2</sub>O in the proportion of 10 lbs of compressed bakers' yeast to 5 liters of H<sub>2</sub>O. Add 2 liters of toluene and stir thoroly at frequent intervals during the first 24 hours. Allow to stand for 7 days with occasional stirring and filter by gravity thru large fluted papers. Mix the residue with 2 liters of H<sub>2</sub>O, filter, and combine the filtrates. Purify<sup>13</sup> by adding 15 g of neutral Pb acetate to each liter of extract and filtering on paper after all Pb acetate has been dissolved. Complete the purification immediately by dialysis or by washing on the ultrafilter as directed under (3).

(2) *Collodion ultrafilter.*<sup>14</sup>—Dissolve 6 g of soluble (in alcohol and ether mixture) pyroxilin or nitro-cellulose such as Astoria's in a mixture of 50 cc of absolute alcohol and 50 cc of absolute ether by first adding the alcohol to the cotton, allowing the mixture to stand in a stoppered flask for 10 min., adding the ether, and shaking. Allow the soln to stand overnight, pour about 100 cc into a 2000 cc cylinder, and coat the entire inside surface of the cylinder with the collodion. Drain, and dry for 10 min. Fill with H<sub>2</sub>O, let stand 10–15 min., pour out the H<sub>2</sub>O, and remove the collodion sack. Test for leaks by filling with H<sub>2</sub>O. Slit open longitudinally and cut out a circular piece about 7–8 inches in diameter. Cut the bottom from a 2 liter bottle or Erlenmeyer flask and grind the edge smooth. Place it upon the still moist collodion disk, fold the edge of the disk up around the bottle, and cement it thereto with collodion that contains an increased percentage of ether. Place 3 or 4 thicknesses of wet filter paper in an 8 inch Büchner funnel. Place the bottle with the collodion membrane upon the filter paper. Pour melted vaseline, to the depth of an inch, between the bottle and inside of the funnel. Provide the bottle with a small mechanical stirring device.

(3) *Washing and concentration of invertase soln by ultrafiltration.*—Filter 4 liters of the partially purified soln thru the ultrafilter, stirring continuously, until about 1 liter remains. Wash with distilled H<sub>2</sub>O introduced by means of a constant level device until the filtrate is colorless, 3 or 4 liters of wash H<sub>2</sub>O being required. During the entire process the invertase soln should be preserved with toluene.

(4) *Activity of invertase soln.*—The following test for activity of the invertase soln is usually adequate: Dilute 1 cc of the invertase preparation to 200 cc. Transfer 10 g of sucrose (granulated sugar) to a sugar flask graduated at 100 cc and 110 cc, dissolve in about 75 cc of H<sub>2</sub>O, add 2 drops of glacial acetic acid, and dilute to the 100 cc mark. To the 100 cc of sugar soln add 10 cc of the dilute invertase soln and mix thoroly and rapidly, noting the exact time at which the solns are mixed. At the termination of exactly 60 min. make a portion of the soln just distinctly alkaline to

litmus paper with anhydrous  $\text{Na}_2\text{CO}_3$  and polarize in a 200 mm tube at  $20^\circ$ . If the invertase soln is sufficiently active, the alkaline soln will polarize approximately  $31^\circ$  Ventzke without correcting for the dilution to 110 cc and the optical activity of the invertase soln.

If more exact information concerning the activity of the invertase preparation is desired, determine its velocity constant as follows: Dilute 1 cc of the invertase soln to 200 cc at  $20^\circ$ ; place in a constant temp. bath at  $20^\circ$ ; and when the soln has attained the latter temp., pipet 20 cc of it into a flask containing 200 cc of a sucrose soln (10 g per 100 cc concentration) that has been previously made distinctly acid to methyl red (corresponding to pH approximately 4.6<sup>15</sup>) by the addition of acetic acid and also brought to a temp. of  $20^\circ$  in the same bath. Mix thoroly and promptly and note the time at which the invertase soln was added. Keep the sucrose-invertase mixture in the constant temp. bath; remove portions at the end of 15, 30, and 45 min.; render each portion just distinctly alkaline to litmus paper with anhydrous  $\text{Na}_2\text{CO}_3$  immediately after removing; and polarize at  $20^\circ$ . Correct all polarizations for the polarization of the invertase soln. Calculate the velocity constant,  $k$ , for each of the polarizations (at the time  $t$ ) subsequent to the initial polarization by the following formula:

$$k = \frac{\log_{10} 1.32 R_0 - \log_{10} (R_t + 0.32 R_0)}{t}, \text{ in which}$$

$k$  = the unimolecular reaction velocity constant;

$t$  = number of min. elapsing from the time the invertase and sucrose solns were mixed until inversion was stopped by addition of  $\text{Na}_2\text{CO}_3$ .

$R_0$  = initial polarization (calculated by multiplying the polarization of the sucrose soln by 10/11 and correcting for the polarization of the invertase soln); and

$R_t$  = polarization at time  $t$ .

An invertase soln of sufficient activity<sup>15</sup> should yield an average value for  $k$  (for the various time periods) of at least 0.1 after multiplying the  $k$  value directly obtained by 200, in order to correct for the initial dilution of the invertase soln. The dilution of the invertase soln above mentioned is made solely for the purpose of determining its activity; the original, undiluted invertase soln is used as the inverting reagent in the determination of sucrose (22) unless the activity of the original invertase soln greatly exceeds a  $K$  value of 0.1, and it is desirable to conserve the invertase. In this case, dilute to a  $K$  value of 0.1, which is done in the same manner as diluting other solns to a standard strength. The activity of the invertase preparation required for rapid inversion, 22(c), is the same as that needed for overnight inversion, 22(b), but the proportion of invertase preparation used in the former case is twice that used in the latter instance.

## 22

### DETERMINATION

(a) *Direct reading.*—Dissolve the double normal weight of the substance (52 g), or a fraction thereof, in  $\text{H}_2\text{O}$  in a 200 cc volumetric flask; add the necessary clarifying agent, 18(a), (b), (d), or (e), avoiding any excess; shake, dilute to the mark with  $\text{H}_2\text{O}$ , mix well, and filter, keeping the funnel covered with a watch-glass. Reject the first 25 cc of the filtrate. If a Pb clarifying agent was used, remove the excess Pb from the soln when sufficient filtrate has collected by adding anhydrous  $\text{Na}_2\text{CO}_3$ , a little at a time, avoiding any excess; mix well and filter again, rejecting the first 25 cc of the filtrate. (Instead of weighing 52 g into a 200 cc flask, two 26 g portions

may be diluted to 100 cc each, and treated exactly as described. Depending on the color of the product, multiples or fractions of the normal weight may be used, and the results reduced by calculation to the basis of 26 g in 100 cc.) Pipet one 50 cc portion of the Pb-free filtrate into a 100 cc flask, dilute with H<sub>2</sub>O to the mark, mix well, and polarize in a 200 mm tube. The result, multiplied by 2, is the direct reading (*P* of formula given below) or polarization before inversion. (If a 400 mm tube is used, the reading equals *P*.) If there is a possibility of mutarotation, proceed as directed under 20.

(b) *Invert reading*.—First determine the quantity of acetic acid necessary to render 50 cc of the Pb-free filtrate distinctly acid to methyl red indicator, 21(4); then to another 50 cc of the Pb-free soln in a 100 cc volumetric flask, add the requisite quantity of acid and 5 cc of the invertase preparation, fill the flask with H<sub>2</sub>O nearly to 100 cc, and let stand overnight (preferably at a temp. not less than 20°). Cool, and dilute to 100 cc at 20°. Mix well and polarize at 20° in a 200 mm tube. If in doubt as to the completion of the hydrolysis, allow a portion of the soln to remain for several hours and again polarize. If there is no change from the previous reading, the inversion is complete. Carefully note the reading and temp. of the soln. If it is necessary to work at a temp. other than 20°, which is permissible within narrow limits, complete the volumes and make both direct and invert readings at the same temp. Correct the polarization for the optical activity of the invertase soln and multiply by 2. Calculate the percentage of sucrose by the following formula:

$$S = \frac{100(P - I)}{142.1 + 0.073(m - 13) - t \cdot 2}, \text{ in which}$$

*S* = percentage of sucrose;

*P* = direct reading, normal soln;

*I* = invert reading normal soln;

*t* = temp. at which readings are made; and

*m* = g of total solids in 100 cc of the invert soln read in the polariscope. Determine the total solids as percentage by weight, as directed under 7, and multiply this figure by the density at 20°, as obtained from XLII, 3.

(c) *Rapid inversion at 55-60°*.<sup>37</sup>—If more rapid inversion is desired, proceed as follows: Prepare the sample as directed under (a) and to 50 cc of the Pb-free filtrate in a 100 cc volumetric flask add glacial acetic acid in sufficient quantity to render the soln distinctly acid to methyl red, 21(4). The quantity of acetic acid required should be determined before pipetting the 50 cc portion, as described under 22(b). Then add 10 cc of invertase soln, mix thoroly, place the flask in a water bath at 55-60°, and allow to stand at that temp. for 15 min. with occasional shaking. Cool, add Na<sub>2</sub>CO<sub>3</sub> until distinctly alkaline to litmus paper, dilute to 100 cc at 20°, mix well, and determine the polarization at 20° in a 200 mm tube. Allow the soln to remain in the tube for 10 min. and again determine the polarization. If there is no change from the previous reading, the mutarotation is complete. Carefully note the reading and the temp. of the soln. Correct the polarization for the optical activity of the invertase soln and multiply by 2. Calculate the percentage of sucrose by the formula given under (b).

If the soln has been rendered so alkaline as to cause destruction of sugar, the polarization, if negative, will in general decrease, since the decomposition of fructose ordinarily is more rapid than that of the other sugars present. If the soln has not been made sufficiently alkaline to complete mutarotation quickly, the polarization,

if negative, will in general increase. As the analyst gains experience he may omit the polarization after 10 min. if he has satisfied himself that he is adding  $\text{Na}_2\text{CO}_3$  in sufficient amount to complete mutarotation at once without causing any destruction of sugar during the period intervening before polarization.

23 *11. By Polarization Before and After Inversion with Hydrochloric Acid<sup>18</sup>—Official*

(In the presence of much levulose, as in honeys, fruit products, sorghum sirup, cane sirup, and molasses, the optical method for sucrose, requiring hydrolysis by acid, gives erroneous results.)

(a) *Direct reading.*—Proceed as directed under 22(a).

(b) *Invert reading.*—Pipet a 50 cc portion of the Pb-free filtrate into a 100 cc flask and add 25 cc of  $\text{H}_2\text{O}$ . Then add, little by little, while rotating the flask, 10 cc of  $\text{HCl}$  (sp. gr. 1.1029 at  $20/4^\circ$  or 24.85° Brix at  $20^\circ$ ). Heat a water bath to  $70^\circ$  and regulate the burner so that the temp. of the bath remains approximately at that point. Place the flask in the water bath, insert a thermometer, and heat with constant agitation until the thermometer in the flask indicates  $67^\circ$ . (This preliminary heating period should require from  $2\frac{1}{2}$ – $2\frac{3}{4}$  min.) From the moment the thermometer in the flask indicates  $67^\circ$ , leave the flask in the bath for exactly 5 min. longer, during which time the temp. should gradually rise to about  $69.5^\circ$ . Plunge the flask at once into  $\text{H}_2\text{O}$  at  $20^\circ$ . When the contents have cooled to about  $35^\circ$ , remove the thermometer from the flask, rinse it, and fill almost to the mark. Leave the flask in the bath at  $20^\circ$  for at least 30 min. longer and finally make up exactly to volume. Mix well and polarize the soln in a 200 mm tube provided with a lateral branch and a water jacket, maintaining a temp. of  $20^\circ$ . This reading must also be multiplied by 2 to obtain the invert reading. If it is necessary to work at a temp. other than  $20^\circ$ , which is permissible within narrow limits, the volumes must be completed and both direct and invert polarizations must be made at exactly the same temp.

Calculate sucrose by the following formula:

$$S = \frac{100 (P - I)}{143 + 0.0676 (m - 13) - t/2}, \text{ in which}$$

$S$  = percentage of sucrose;

$P$  = direct reading, normal soln;

$I$  = invert reading, normal soln;

$t$  = temp. at which readings are made; and

$m$  = g of total solids in 100 cc of the invert soln read in the polariscope.

Determine the total solids as percentage by weight, as directed under 7, and multiply this figure by the density at  $20^\circ$  as obtained from XLII, 3.

(c) *Inversion at room temperature.*—The inversion may also be accomplished as follows: (1) To 50 cc of the clarified soln, freed from Pb, add 10 cc of  $\text{HCl}$  (sp. gr. 1.1029 at  $20/4^\circ$  or 24.85° Brix at  $20^\circ$ ) and set aside for 24 hours at a temp. not below  $20^\circ$ ; or, (2) if the temp. is above  $25^\circ$ , set aside for 10 hours. Make up to 100 cc at  $20^\circ$  and polarize as directed under (b). Under these conditions the formula must be changed to the following:

$$S = \frac{100 (P - I)}{143.2 + 0.0676 (m - 13) - t/2}.$$

SUCROSE AND RAFFINOSE<sup>1,2</sup>*I. By Polarization Before and After Treatment with Two Enzyme Preparations—Official, first action*

## 24

## REAGENTS

(a) *Invertase soln (top yeast extract).*—Prepare as directed under 21. This soln should be free from the enzyme melibiase. Its invertase activity should be at least as great as that used for the determination of sucrose in the absence of raffinose, 21(4).

(b) *Invertase-melibiase soln (bottom yeast extract).*—Prepare as directed under 21, using bottom fermenting yeast (brewers' yeast) instead of bakers' yeast. The invertase activity should be at least as great as in (a).

Test the melibiase activity of the soln as follows: Add 2 cc of the soln to be tested to 20 cc of a weakly acid melibiose soln polarizing  $+20.0^{\circ}\text{V}$  and allow to stand 30 min. at about  $20^{\circ}$ . Then add sufficient  $\text{Na}_2\text{CO}_3$  to render the soln slightly alkaline to litmus paper. A preparation suitable for the overnight hydrolysis of solns containing not more than 0.2 g of raffinose in 100 cc should have hydrolyzed 35% of the melibiose present under the conditions mentioned; a preparation suitable for the overnight hydrolysis of solns containing not more than 0.65 g of raffinose in 100 cc should have produced 50% hydrolysis of melibiose; and a preparation suitable for the overnight hydrolysis of solns containing 0.65–1.3 g of raffinose in 100 cc should have hydrolyzed at least 70% of the melibiose present under the above condition. The polarizations that correspond to 35, 50 and 70% hydrolysis of a melibiose soln polarizing, before hydrolysis,  $+20^{\circ}\text{V}$  are:  $+16.4^{\circ}$ ,  $+14.9^{\circ}$  and  $+12.9^{\circ}\text{V}$ , respectively.

## 25

## DETERMINATION

In the analysis of sugar beet products, weigh the quantity of material specified in the following table, transfer to a 300 cc volumetric flask, add the quantity of basic

*Quantities of sample and reagents required for clarification and deleading of beet sugar-house products*

MATERIAL	QUANTITY PER 100 cc	BASIC LEAD ACETATE (55° BRHX)	AMMONIUM DIHYDROGEN PHOSPHATE
	grams	cc	gram
Cossettes <sup>a</sup> .....	13	3	0.2
Pulp .....	100 cc <sup>b</sup>	2-4	0.2
Lime cake or sewer <sup>c</sup> .....	26.5	1.5	..... <sup>d</sup>
Thin juice .....	52	2	0.2-0.3
Thick juice .....	26	4	0.3-0.4
White massecuite .....	13 or 26	3 or 6	0.3-0.7
High wash sirup .....	13 or 26	3 or 6	0.3-0.7
High green sirup .....	13 or 26	5 or 10	0.3-0.7
Raw or remelt massecuite .....	13	6	0.3-0.4
Raw or remelt sugar .....	26	3-4	0.3-0.4
Sugar melter .....	26	2-3	0.3-0.4
Low wash sirup .....	13	8-10	0.4-0.5
Low green sirup or molasses .....	13	10	0.4-0.5
Saccharate cakes and milk (carbonated) .....	26	4-6	0.3-0.4
Steffen waste and wash waters <sup>e</sup> .....	78 or 50 cc	2-3	0.2

<sup>a</sup> Usual method of extraction, 26 g in 201.2 cc.

<sup>b</sup> Dilute to 110 cc.

<sup>c</sup> Neutralize with acetic acid before adding basic Pb acetate.

<sup>d</sup> Lime in soln will be partly precipitated by the phosphate, and it is necessary to add sufficient phosphate to complete the precipitation of both the lead and lime salts; hence no definite quantity can be specified.

Pb acetate soln indicated in the table, and dilute to volume at 20°. Mix thoroly and filter thru fluted paper in a closely covered funnel, rejecting the first 25 cc of filtrate. When sufficient filtrate has collected, remove the Pb from the soln by adding  $\text{NH}_4\text{H}_2\text{PO}_4$  in as small excess as possible (see table). This condition is readily determined after a little practice by the appearance of the  $\text{Pb}_3(\text{PO}_4)_2$  precipitate, which usually flocculates and settles rapidly in the presence of a slight excess of the salt. Mix well and filter, again rejecting at least the first 25 cc of the filtrate. Make a direct polarization in a 200 mm tube at 20° unless the soln contains an appreciable quantity of invert sugar, in which case pipet a 50 cc portion of the Pb-free filtrate into a 100 cc flask, dilute with  $\text{H}_2\text{O}$  to the mark, mix well, and polarize at 20°, preferably in a 400 mm tube. This reading, calculated to the normal weight of 26 g in 100 cc and 200 mm tube length, is the direct reading ( $P$ ) of the formula given below for polarization before inversion.

Transfer two 50 cc portions of the Pb-free filtrate to 100 cc flasks. To one add 5 cc of invertase soln (top yeast extract) and to the other 5 cc of invertase-melibiose soln (bottom yeast extract), let stand overnight at atmospheric temp. (preferably not below 20°), dilute to volume, mix well, and polarize at 20°, preferably in a 400 mm jacketed tube. If a rapid hydrolysis is desired, add 10 cc of each of the enzyme solns to the 50 cc portions of delead filtrate in 100 cc flasks and place in a water bath at 50–55° for 40 min. Then add  $\text{Na}_2\text{CO}_3$  until the soln is slightly alkaline to litmus paper, dilute to volume at 20°, mix well, and polarize at 20°, preferably in a 400 mm tube. Correct the invert readings for the optical activity of the enzyme soln and calculate the polarization to that of a normal weight soln of 26 g in 100 cc; also calculate the reading to a 200 mm tube length, if necessary.

Calculate the percentages of anhydrous raffinose and sucrose from the following formulas:

$$R = 1.354 (A - B);$$

$$S = \frac{(P - 2.202A + 1.202B) 100}{132.12 - 0.00718 [132.12 - (P - 2.202A + 1.202B)]}, \text{ in which}$$

$$R = \text{percentage of raffinose};$$

$$S = \text{percentage of sucrose};$$

$$P = \text{direct polarization, normal soln};$$

$$A = \text{corrected polarization after top yeast hydrolysis, normal soln; and}$$

$$B = \text{corrected polarization after bottom yeast hydrolysis, normal soln.}$$

The quantities  $A$  and  $B$  are treated algebraically.

## 26 II. By Polarization Before and After Inversion with $\text{HCl}$ —Official

(Of value chiefly in the analysis of beet products.)

If the direct reading is more than 1° higher than the percentage of sucrose as calculated by the formula given under 23(b), raffinose is probably present. Calculate sucrose and raffinose by the following formulas:<sup>29</sup>

When the polarizations are made at 20°:

$$S = \frac{0.514P - I}{0.844} \text{ and } R = \frac{0.33P + I}{1.563}, \text{ in which}$$

$$P = \text{direct reading, normal soln};$$

$$I = \text{invert reading, normal soln};$$

$$S = \text{percentage of sucrose; and}$$

$$R = \text{percentage of anhydrous raffinose.}$$



The following formulas<sup>20</sup> are applicable at all temps. other than 20°:

$$S = \frac{P(0.478 + 0.0018t_2) - I(1.006 - 0.0003t_1)}{(0.908 - 0.0032t_2)(1.006 - 0.0003t_1)}, \text{ and}$$

$$R = \frac{P(0.43 - 0.005t_2) + I(1.006 - 0.0003t_1)}{(1.681 - 0.0059t_2)(1.006 - 0.0003t_1)} \text{ in which}$$

$P$  = direct reading, normal soln;

$I$  = invert reading, normal soln;

$S$  = percentage of sucrose

$R$  = percentage of anhydrous raffinose; and

$t_1$  = temp. of the direct polarization; and

$t_2$  = temp. of the invert polarization.

## 27

SUCROSE BY DOUBLE DILUTION METHOD<sup>21</sup>—OFFICIAL

(Substances in which the volume of the combined insoluble matter and precipitate from clarifying agents is more than 1 cc from 26 g.)

Weigh a half-normal weight of the sample and dilute the soln to 100 cc, using the appropriate clarifier (basic Pb acetate for dark colored confectionery or molasses and alumina cream for light colored confectionery). Also weigh a normal weight of the sample and dilute a second soln with the clarifier to 100 cc. Filter, and obtain direct polariscopic readings of both solns. Invert each soln as directed under 22(b) or (c) or 23(b) or (c) and obtain the respective invert readings.

The true direct polarization of the sample = 4 times the direct polarization of the diluted soln less the direct polarization of the undiluted soln.

The true invert polarization = 4 times the invert polarization of the diluted soln less the invert polarization of the undiluted soln.

Calculate the sucrose from the true polarizations thus obtained, using the formula under 22 or 23 corresponding to the method of inversion used.

## SUCROSE—CHEMICAL METHODS

## 28

*From Reducing Sugars Before and After Inversion*—Official

Determine the reducing sugars (clarification having been effected with neutral Pb acetate, never with basic Pb acetate) as directed under 38 and calculate to invert sugar from XLII, 9. Invert the soln as directed under 22(b) or (c), or 23(b) or (c); exactly neutralize the acid; and again determine the reducing sugars, but calculate them to invert sugar from the table referred to above, using the invert sugar column alone. Deduct the percentage of invert sugar obtained before inversion from that obtained after inversion and multiply the difference by 0.95 to obtain the percentage of sucrose. The solns should be diluted in both determinations so that not more than 240 mg of invert sugar is present in the quantity taken for reduction. It is important that all Pb be removed from the soln with anhydrous powdered K oxalate or Na<sub>2</sub>CO<sub>3</sub> before reduction.

COMMERCIAL GLUCOSE<sup>22</sup> (APPROXIMATE) POLARIMETRIC METHODS

## 29

*Method 1.* Official

(Substances containing little or no invert sugar.)

Commercial glucose cannot be determined accurately owing to the varying quantities of dextrin, maltose, and dextrose present in the product. However, in sirups

in which the quantity of invert sugar is so small as not to affect appreciably the result, commercial glucose may be estimated approximately by the following formula:

$$G = \frac{(a-S) 100}{211}, \text{ in which}$$

$G$  = percentage of commercial glucose solids;

$a$  = direct polarization, normal soln; and

$S$  = percentage of cane sugar.

Express the results in terms of commercial glucose solids polarizing  $+211^{\circ}\text{V}$ . (This result may be recalculated in terms of commercial glucose of any Baumé reading desired.)

30

#### Method II.—Official

(Substances containing invert sugar.)

Prepare an inverted half-normal soln of the substance as directed under 23(b), except to cool the soln after inversion, make neutral to phenolphthalein with NaOH soln, slightly acidify with HCl (1+5), and treat with 5–10 cc of alumina cream before making up to the mark. Filter, and polarize at  $87^{\circ}$  in a 200 mm jacketed metal tube, preferably silver. Multiply the reading by 200 and divide by the factor 196 to obtain the quantity of commercial glucose solids polarizing  $+211^{\circ}\text{V}$ . (This result may be recalculated in terms of commercial glucose of any Baumé reading desired.)

#### INVERT SUGAR—CHEMICAL METHODS

##### I. Lane-Eynon General Volumetric Method<sup>23</sup>—Tentative

31

#### REAGENTS

*Soxhlet's modification of Fehling's soln.*—Prepare by mixing immediately before use equal volumes of (a) and (b).

(a) *Copper sulfate soln.*—Dissolve 34.639 g of  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  in  $\text{H}_2\text{O}$ , dilute to 500 cc, and filter thru prepared asbestos.

(b) *Alkaline tartrate soln.*—Dissolve 173 g of Rochelle salts and 50 g of NaOH in  $\text{H}_2\text{O}$ , dilute to 500 cc, allow to stand for 2 days, and filter thru prepared asbestos.

32

#### STANDARDIZATION AND METHOD OF TITRATION

Pipet accurately 10 or 25 cc of mixed Soxhlet's reagent or pipet 5 or 12.5 cc of each of Soxhlet's solns (a) and (b) into a flask of 300–400 cc capacity. The quantity of Cu taken will differ slightly between the 2 methods of pipetting, and the method used must be carried out consistently during standardization and determination. Prepare a standard soln of the pure sugar of such concentration that more than 15 cc and less than 50 cc will be required to reduce all the Cu. The titer may be calculated as follows:  $\frac{\text{factor}}{\text{mg sugar in 1 cc}}$ . Add almost the whole of the sugar soln

required to effect reduction of all the Cu, so that not more than 0.5–1 cc is required later to complete the titration. Heat the cold mixture to boiling on a wire gauze and maintain in moderate ebullition for 2 min., lowering the flame sufficiently to avoid bumping. Without removing the flame add 2–5 drops of 1% aqueous methylene blue soln and complete the titration within a total boiling time of about 3 min. by small additions of sugar soln to decolorization of the indicator.

Multiply the titer by the number of mg in 1 cc of the standard soln to obtain the factor. Compare with the tabulated factor to determine the correction, if any, to be applied to the table. Small deviations from the tabulated factors may arise from variations in individual procedure or composition of reagents. If only approximate results (within 1%) are required the standardization may be omitted, provided the specifications of the analysis are rigidly observed.

## 33

## DETERMINATION

If the approximate concentration of the sugar in the sample is unknown, proceed by the incremental method of titration. Add to 10 or 25 cc of Soxhlet's soln 15 cc of the sugar soln and heat to boiling over a wire gauze. Boil about 15 seconds and add rapidly further quantities of the sugar soln until only the faintest perceptible blue color remains. Then add 2-5 drops of methylene blue and complete the titration by adding the sugar soln dropwise. (The error resulting from this titration will not generally exceed 1%.)

For higher precision repeat the titration, adding almost the whole of the sugar soln required to reduce all the Cu and proceed as directed above. In Table 15, XLII, find the factor corresponding to the titer and apply the correction previously determined. Estimate as follows:

$$\frac{\text{factor} \times 100}{\text{titer}} = \text{mg of sugar in 100 cc.}$$

*II. Seales Method?— Tentative*

(Suitable when very small quantities of sugar are present.)

## 34

## REAGENT

*Benedict's solns.*—Dissolve 16 g of  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  in 125-150 cc of  $\text{H}_2\text{O}$ . Then dissolve 150 g of Na citrate, 130 g of  $\text{Na}_2\text{CO}_3$  (anhydrous), and 10 g of  $\text{NaHCO}_3$  in about 650 cc of  $\text{H}_2\text{O}$ , heating to accelerate soln. Combine the 2 solns with stirring. Cool, make to 1 liter, and filter.

## 35

## PROCEDURE

Transfer 20 cc of the Cu reagent to a 300 cc Erlenmeyer flask fitted with a 2-holed rubber stopper. Add 10 cc of sugar soln containing less than 20 mg of reducing sugar. Place over a flame, bring to boiling in 4 min., and continue the boiling for exactly 3 min. (Approximate conditions, flame 50 mm, cone 20 mm, asbestos gauze 30 mm above burner. If preferred, an electric hot plate may be used, in which case a period of 5 min. is required to raise the soln to the boiling point.) At the expiration of 3 min. from the beginning of the boiling, cool rapidly by holding under a cold water faucet, add 100 cc of acetic acid soln (24 cc of glacial acetic acid per liter) from a graduate, and transfer an exactly measured amount of 0.04 N I soln. Add 25 cc of HCl (60 cc per liter) from a pipet held against the side of the flask, and agitate to distribute the acid rapidly. Rotate the flask for 1 min. to insure the soln of all  $\text{Cu}_2\text{Cl}_2$ . Titrate excess I with 0.04 N thiosulfate soln, using starch soln as an indicator.

For amounts less than 20 mg of sugar each cc of thiosulfate will represent a constant quantity of sugar; for dextrose, approximately 1.12 mg per cc. (For accurate work the analyst should determine the conversion factor for the particular conditions under which he is working by using control solns of the pure sugars under examination.)

III. Munson and Walker General Method<sup>25</sup>—Official

36

## REAGENTS

*Asbestos*.—Digest the asbestos, which should be the amphibole variety, with HCl (1+3) for 2-3 days. Wash free from acid, digest for a similar period with 10% NaOH soln, and then treat for a few hours with hot alkaline tartrate soln (old alkaline tartrate solns that have stood for some time may be used for this purpose) of the strength used in sugar determinations. Wash the asbestos free from alkali; digest for several hours with HNO<sub>3</sub> (1+3); and, after washing free from acid, shake with H<sub>2</sub>O into a fine pulp. In preparing the Gooch crucible, make a film of asbestos  $\frac{1}{4}$  inch thick and wash thoroly with H<sub>2</sub>O to remove fine particles of asbestos. If the precipitated Cu<sub>2</sub>O is to be weighed as such, wash the crucible with 10 cc of alcohol, then with 10 cc of ether; dry for 30 min. at 100°, cool in a desiccator, and weigh.

The other reagents and solns used are described under 31.

37

## PRECIPITATION OF CUPROUS OXIDE

Transfer 25 cc of each of the CuSO<sub>4</sub> and alkaline tartrate solns to a 400 cc beaker of alkali-resistant glass and add 50 cc of the reducing sugar soln, or if a smaller volume of sugar soln is used, add H<sub>2</sub>O to make the final volume 100 cc. Heat the beaker on an asbestos gauze over a Bunsen burner, regulate the flame so that boiling begins in 4 min., and continue the boiling for exactly 2 min. (It is important that these directions be strictly observed. To regulate the burner for this purpose it is advisable to make preliminary tests, using 50 cc of the reagent and 50 cc of H<sub>2</sub>O before proceeding with the actual determination.) Keep the beaker covered with a watch-glass during the heating. Filter the hot soln at once thru an asbestos mat in a porcelain Gooch crucible, using suction. Wash the precipitate of Cu<sub>2</sub>O thoroly with H<sub>2</sub>O at a temp. of about 60° and either weigh directly as Cu<sub>2</sub>O as directed under 38, or determine the quantity of reduced Cu by one of the methods described under 38-42, respectively. Conduct a blank determination, using 50 cc of the reagent and 50 cc of H<sub>2</sub>O, and if the weight of Cu<sub>2</sub>O obtained exceeds 0.5 mg, correct the result of the reducing sugar determination accordingly. The alkaline tartrate soln deteriorates on standing, and the quantity of Cu<sub>2</sub>O obtained in the blank increases.

## DETERMINATION OF REDUCED COPPER

38

*Direct Weighing of Cuprous Oxide*

(Use only for determinations in solns of reducing sugars of comparatively high purity. In products containing large quantities of mineral or organic impurities, including sucrose, determine the Cu of the Cu<sub>2</sub>O by one of the methods described under 38-42, since the Cu<sub>2</sub>O is very likely to be contaminated with foreign matter.)

Prepare a Gooch crucible as directed under 36. Collect the precipitated Cu<sub>2</sub>O on the mat as directed under 37 and wash thoroly with hot H<sub>2</sub>O, then with 10 cc of alcohol, and finally with 10 cc of ether. Dry the precipitate for 30 min. in a water oven at the temp. of boiling H<sub>2</sub>O, cool, and weigh. Calculate the weight of metallic Cu, using the factor 0.8882. Obtain from XLII, 9, the weight of invert sugar equivalent to the weight of Cu.

The number of mg of Cu reduced by a given quantity of reducing sugar varies, depending upon whether or not sucrose is present. In the tables the absence of sucrose is assumed except in the entries under invert sugar, where, in addition to the column for invert sugar alone, one column is given for mixtures of invert sugar and

sucrose containing 0.4 g of total sugar in 50 cc of soln and one column for invert sugar and sucrose when the 50 cc of soln contains 2 g of total sugar. Two entries are also given under lactose and sucrose mixtures, showing proportions of 1 part lactose to 4 and 12 parts of sucrose, respectively.

*Volumetric Thiosulfate Method<sup>28</sup>—Official, first action*

39

## REAGENT

*Standard thiosulfate soln.*—Prepare a soln containing 39 g of pure  $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$  in 1 liter. Weigh accurately 0.2–0.4 g of pure Cu and transfer to a 250 cc Erlenmeyer flask roughly graduated by marks at 20 cc intervals. Dissolve the Cu in 5 cc of a mixture of equal volumes of  $\text{HNO}_3$  and  $\text{H}_2\text{O}$ , dilute to 20 or 30 cc, boil to expel the red fumes, add a slight excess of strong Br water, and boil until the Br is completely driven off. Cool, and add NaOH soln with agitation until a faint turbidity of  $\text{Cu}(\text{OH})_2$  appears (about 7 cc of a 25% NaOH soln is required). Discharge the turbidity with a few drops of acetic acid and add 2 drops in excess. Prepare a soln of 42 g of KI in 100 cc of soln made very slightly alkaline to avoid formation of I<sub>2</sub> and its oxidation.

It is essential for the thiosulfate titration that the concentration of KI in the soln be carefully regulated. If the soln contains less than 320 mg of Cu, at the completion of the titration 4.2–5 g of KI should have been added for each 100 cc of total soln. If greater quantities of Cu are present, add the KI soln slowly from a buret with constant agitation in amounts proportionately greater.

Observe the volume of the Cu soln and add 1 cc of KI soln for each 10 cc of the soln undergoing titration. Titrate at once with the thiosulfate soln until the brown color becomes faint. Again observe the volume and add an additional volume of KI to make the required concentration, noting from the volume of the thiosulfate the approximate Cu content of the soln. Add sufficient starch indicator, VI, 3(e), to produce a marked blue coloration. Continue the titration cautiously until the color changes toward the end to a faint lilac. As the end point is approached, add the thiosulfate in fractions of drops, allowing the precipitate to settle slightly after each addition. 1 cc of the thiosulfate soln = about 10 mg of Cu.

40

## DETERMINATION

Wash the precipitated  $\text{Cu}_2\text{O}$ , cover the Gooch crucible with a watch-glass, and dissolve the oxide by means of 5 cc of  $\text{HNO}_3$  (1+1) directed under the watch-glass with a pipet. Collect the filtrate in a 250 cc Erlenmeyer flask which is roughly graduated by marks at 20 cc intervals and wash the watch-glass and Gooch crucible free from Cu. Proceed as directed under 39, beginning with "boil to expel the red fumes . . ."

41

*Volumetric Permanganate Method*

Filter and wash the  $\text{Cu}_2\text{O}$  as directed under 37. Transfer the asbestos film to the beaker, add about 30 cc of hot  $\text{H}_2\text{O}$ , and heat the precipitate and asbestos thoroly. Rinse the crucible with 50 cc of a hot saturated soln of  $\text{Fe}_2(\text{SO}_4)_3$  in 20%  $\text{H}_2\text{SO}_4$ , receiving the rinsings in the beaker containing the precipitate. After the  $\text{Cu}_2\text{O}$  is dissolved, wash the soln into a large Erlenmeyer flask and immediately titrate with a standard soln of  $\text{KMnO}_4$ , 1 cc of which should be equivalent to 0.010 g of Cu. Standardize this soln by making 6 or more determinations with the same sugar soln, titrating  $\frac{1}{2}$  of the precipitates obtained and determining the Cu in the others by electrolysis. The average weight of Cu obtained by electrolysis, divided by the average

number of cc of  $\text{KMnO}_4$  soln required for the titrations, gives the weight of Cu equivalent to 1 cc of the standard  $\text{KMnO}_4$  soln. A soln standardized with iron or oxalic acid will give too low a result, but agreement with electrolytic determination will be obtained if pure Na oxalate is used and is made acid with  $\text{H}_2\text{SO}_4$ .

42 *Electrolytic Deposition from Sulfuric and Nitric Acid Solution*<sup>27</sup>

Decant the hot soln thru an asbestos mat in a Gooch crucible; wash the beaker and precipitate thoroly with hot  $\text{H}_2\text{O}$ . Transfer the asbestos film from the crucible to the beaker by means of a glass rod, and rinse the crucible with about 30 cc of a boiling mixture containing 65 cc of  $\text{H}_2\text{SO}_4$  and 50 cc of  $\text{HNO}_3$  per liter. Heat, and agitate until soln is complete and the oxides of N have been volatilized. Filter, and transfer to a weighed Pt dish. Dilute to about 100 cc and deposit the Cu by electrolysis at 20–30°, using about 2.5 volts and a current density of about 0.5 ampere. Cover the dish with a split watch-glass to avoid loss by spattering. Electrolysis requires about 14 hours, but it may be allowed to continue overnight. Test 1 cc with  $\text{H}_2\text{S}$  soln for complete deposition. Wash thoroly with  $\text{H}_2\text{O}$ ; then break the current, wash with alcohol and ether successively, dry at about 50°, and weigh.

If preferred the electrolysis may be conducted in a beaker, the Cu being deposited upon a weighed Pt foil or Pt gauze.

IV. *Herzfeld Gravimetric Methods—Official*

43

*Method I.*

(For materials containing 1.5% or less of invert sugar and 98.5% or more of sucrose.)

Prepare the soln of the material to be examined to contain 20 g in 100 cc, clarify with neutral Pb acetate, and remove the excess of Pb with  $\text{Na}_2\text{CO}_3$ . Place 50 cc of the reagent, 31, and 50 cc of the sugar soln in a 250 cc beaker. Heat this mixture at such a rate that approximately 4 min. is required to bring it to the boiling point and boil for exactly 2 min. Add 100 cc of cold recently boiled  $\text{H}_2\text{O}$ . Filter immediately thru asbestos, 36, and determine the Cu by one of the methods described under 38–42. Obtain the corresponding percentage of invert sugar from XLII, 10.

44

*Method II.*

(For materials containing more than 1.5% of invert sugar and less than 98.5% of sucrose.)

Prepare a soln of suitable concentration of the material to be examined, clarify with neutral Pb acetate, and remove the excess of Pb. Prepare a series of solns in large test tubes by adding 1, 2, 3, 4, and 5 cc of this soln to each tube successively. Add 5 cc of the reagent, 31, to each, heat to boiling, boil 2 min., and filter. Note the volume of sugar soln that gives the filtrate lightest in tint, but still distinctly blue. Place 20 times this volume of the sugar soln in a 100 cc flask, dilute to the mark, and mix well. Use 50 cc of the soln for the determination, and proceed as directed under 43. For the calculation of the result use the following formulas and the table of factors of Meissl and Hiller, XLII, 11,

Let  $Cu$  = the weight of Cu obtained;

$P$  = the polarization of the sample;

$W$  = the weight of the sample in the 50 cc of the soln used for the determination; and

$F$  = the factor obtained from the table for the conversion of Cu to invert sugar.

Then  $\frac{Cu}{2} = Z$ , approximate weight of invert sugar;

$Z \times \frac{100}{W} = Y$ , approximate percentage of invert sugar;

$\frac{100 P}{P + Y} = R$ , approximate percentage of sucrose in mixture of sugars;

$100 - R = I$ , approximate percentage of invert sugar;

$\frac{CuF}{W} =$  percentage of invert sugar.

Use the factor  $F$  for calculating  $Cu$  to invert sugar as found in Table 11, XLII.  
*Example:* The polarization of a sugar is 86.4, and 50 cc of soln containing 3.256 g of sample gives 0.290 g of  $Cu$ .

$$\frac{Cu}{2} = \frac{0.290}{2} = 0.145 = Z.$$

$$\frac{Z \times 100}{W} = 0.145 \times \frac{100}{3.256} = 4.45 = Y.$$

$$\frac{100 P}{P + Y} = \frac{8460}{86.4 + 4.45} = 95.1 = R.$$

$$100 - R = 100 - 95.1 = I = 4.9.$$

$$R:I = 95.1:4.9.$$

By consulting the table it will be seen that the vertical column headed 150 is nearest to  $Z$ , 145, and the horizontal column headed 95:5 is nearest to the ratio of  $R$  to  $I$ , 95.1:4.9. Where these columns meet is found the factor 51.2, which enters into the final calculation:

$$\frac{CuF}{W} = \frac{0.290 \times 51.2}{3.256} = 4.56\% \text{ of invert sugar.}$$

#### DEXTROSE—CHEMICAL METHODS

##### 45 *Lane-Eynon General Volumetric Method—Tentative*

Proceed as directed under 33, referring the titer to XLII, 15 or 16.

##### 46 *Munson-Walker General Gravimetric Method—Official*

Proceed as directed under 37 and obtain from XLII, 9, the weight of dextrose equivalent to the weight of  $Cu$  reduced.

#### *Allihn Gravimetric Method—Official*

##### 47

##### REAGENTS

(a) *Copper sulfate soln.*—Dissolve 34.639 g of  $CuSO_4 \cdot 5H_2O$  in  $H_2O$  and dilute to 500 cc.

(b) *Alkaline tartrate soln.*—Dissolve 173 g of Rochelle salts and 125 g of  $KOH$  in  $H_2O$  and dilute to 500 cc.

48

## DETERMINATION

Place 30 cc of the  $\text{CuSO}_4$  soln, 30 cc of the alkaline tartrate soln, and 60 cc of  $\text{H}_2\text{O}$  in a beaker, and heat to boiling. Add 25 cc of the soln of the material to be examined containing not more than 0.25 g of dextrose, and boil exactly 2 min., keeping the beaker covered. Filter immediately thru asbestos and obtain the weight of Cu by one of the methods given under 38-42, respectively. Obtain the corresponding weight of dextrose from XLII, 14.

## LEVULOSE—CHEMICAL METHODS

49

*Lane-Eynon General Volumetric Method*

Proceed as directed under 33, referring the titer to XLII, 15 or 16.

*Jackson-Matthews Modification of Nyns Selective Method<sup>28</sup>—Tentative*

50

## REAGENT

*Ost's soln.*—Dissolve 250 g of  $\text{K}_2\text{CO}_3$  (anhydrous) in about 700 cc of hot  $\text{H}_2\text{O}$ , add 100 g of pulverized  $\text{KHCO}_3$ , and agitate the mixture until completely dissolved. Cool, and add with very vigorous agitation a soln of 25.3 g of  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  in 100-150 cc of  $\text{H}_2\text{O}$ . Make to 1 liter and filter.

51

## PROCEDURE

Transfer 50 cc of Ost's soln to a 150 cc Erlenmeyer flask and add by means of an accurately graduated pipet a volume of the soln to be analyzed that contains not more than 92 mg of levulose or its equivalent of a levulose-dextrose mixture, remembering that dextrose has about one-twelfth the reducing power of levulose. Add enough  $\text{H}_2\text{O}$  to make the total volume 70 cc. Immerse in a water bath, regulated preferably within  $0.1^\circ$ , at  $55^\circ$ . Digest for exactly 75 min., agitating with a rotary motion at intervals of 10 or 15 min.

Filter the precipitated Cu on a closely packed Gooch crucible and wash flask and precipitate thoroly without attempting to transfer the precipitate quantitatively. Determine Cu by one of the methods described under 38-42. As it is usually difficult to transfer the Cu precipitate quantitatively from the Erlenmeyer flask, select a method of Cu analysis in which the total Cu is dissolved in  $\text{HNO}_3$  and determined by electrolysis or thiosulfate titration or in a  $\text{Fe}_2(\text{SO}_4)_3\text{-H}_2\text{SO}_4$  mixture followed by a permanganate titration.

See Table 13, XLII, for the levulose equivalent. If the sample contained glucose in addition to levulose the analytical result is not true but "apparent" levulose, as glucose has an appreciable reducing action under the conditions of the analysis. To determine the correction for glucose analyze the sample also for total reducing sugar and compute the true glucose and levulose by a series of approximations. Calculate the percentage of reducing sugar in the original sample and similarly the percentage of "apparent" levulose. The difference between these two percentages is the "apparent" glucose. Divide the apparent glucose by the factor 12.4 and deduct the result from the apparent levulose to obtain a new approximation to the true levulose. Deduct the new levulose percentage from the total reducing sugar percentage to obtain a more nearly correct value for true glucose and again divide by 12.4. Deduct the quotient from the original value of the "apparent" levulose and continue the approximation in the same manner until the percentage of levulose remains essentially unaltered by two successive approximations.



If the original sample contained sucrose, determine the latter by means of the Clerget procedure, 23. Correct the Cu for the reducing action of sucrose before referring to the table, XLII, 13. 1, 2, 3, 4, and 5 g of sucrose precipitate under the conditions of the analysis 3.3, 5.7, 7.4, 8.5, 9.0 mg of Cu, respectively.

#### MALTOSE—CHEMICAL METHODS

##### 52 *Lane-Eynon General Volumetric Method—Tentative*

Proceed as directed under 33, referring the titer to XLII, 15 or 16.

##### 53 *Munson-Walker General Gravimetric Method—Official*

Proceed as directed under 37 and obtain from XLII, 9, the weight of maltose equivalent to the weight of Cu reduced.

#### Wien Method—Official

##### 54

#### REAGENTS

The reagents and solns used are described under 31 and 36.

##### 55

#### DETERMINATION

Place 50 cc of the reagent, 31, in a beaker and heat to the boiling point. When it is boiling briskly, add 25 cc of the maltose soln containing not more than 0.250 g of maltose and boil for 4 min. Filter immediately thru asbestos, 36, and determine, by one of the methods given under 38–42, respectively, the quantity of Cu reduced. Obtain from XLII, 12, the weight of maltose equivalent to the weight of Cu.

#### LACTOSE—CHEMICAL METHODS

##### 56 *Lane-Eynon General Volumetric Method—Tentative*

Proceed as directed under 33, referring the titer to XLII, 15 or 16.

##### 57 *Munson-Walker General Gravimetric Method—Official*

Proceed as directed under 37 and obtain from XLII, 9, the weight of lactose equivalent to the weight of Cu reduced.

##### 58

#### REDUCING SUGARS OTHER THAN DEXTROSE—OFFICIAL

Proceed as directed under 48 and multiply the weight of dextrose found by the following factors:

Levulose, 1.093; invert sugar, 1.044; arabinose, 0.969; xylose, 1.017; and galactose, 1.114.

#### CONFECTIONERY

##### 59

#### PREPARATION OF SAMPLE—OFFICIAL

If the composition of the entire sample is desired, grind and mix thoroly. If the sample is composed of layers or of distinctly different portions and it is desired to examine these individually, separate with a knife or other mechanical means as completely as possible and grind and mix each portion thoroly.

##### 60

#### MOISTURE—OFFICIAL

Proceed as directed under 2, 3, or 4.

**61** ASH—OFFICIAL

Proceed as directed under 8 or 9.

**62** MINERAL CONSTITUENTS

Proceed as directed under XII.

**63** SOLUBLE AND INSOLUBLE ASH—OFFICIAL

Proceed as directed under 12.

**64** ALKALINITY OF SOLUBLE ASH—OFFICIAL

Proceed as directed under 13.

**65** ALKALINITY OF INSOLUBLE ASH—OFFICIAL

Proceed as directed under 14.

**66** MINERAL ADULTERANTS IN THE ASH—TENTATIVE

Proceed as directed under 15.

**67** NITROGEN—OFFICIAL

Determine N in 2–5 g of the material as directed under II, 21, 23, or 25, using a larger quantity of  $\text{H}_2\text{SO}_4$  if necessary for complete digestion.

## SUCROSE—POLARIMETRIC METHODS

**68** SUCROSE IN THE ABSENCE OF RAFFINOSE

Proceed as directed under 22, 23, or 27.

## SUCROSE—CHEMICAL METHODS

**69** *By Reducing Sugars Before and After Inversion—Official*

Proceed as directed under 28.

**70** COMMERCIAL GLUCOSE—OFFICIAL

Proceed as directed under 29 or 30.

**71** STARCH—TENTATIVE

Measure 25 cc of a soln of uniform mixture (representing 5 g of the sample) into a 300 cc beaker, or introduce 5 g of the finely ground sample (previously extracted with ether if the sample contains much fat) into the beaker; add sufficient  $\text{H}_2\text{O}$  to make the volume 100 cc; heat to about  $60^\circ$  (avoiding if possible gelatinizing the starch); and allow to stand for about an hour, stirring frequently to secure complete soln of the sugars. Transfer to a wide-mouthed bottle, rinse the beaker with a little warm  $\text{H}_2\text{O}$ , and cool. Add an equal volume of 95% alcohol, mix, and allow to stand at least an hour. Centrifuge until the precipitate is closely packed on the bottom of the bottle and decant the supernatant liquid thru a hardened filter. Wash the precipitate with successive 50 cc portions of alcohol, 50% by volume, by centrifuging and decanting thru the filter until 3 or 4 drops of the washings give no test for sugar with alpha-naphthol as described under 122. Transfer the residue from the bottle and the hardened filter to a large flask and determine starch as directed under XXVII, 23.

## ETHER EXTRACT

72

*I. Continuous Extraction Method—Tentative*

(1) Measure 25 cc of a 20% mixture or soln into a very thin, readily frangible glass evaporating shell, or a thin lead or tin foil dish containing 5–7 g of freshly ignited asbestos fiber; or (2) if possible to obtain a uniform sample, weigh 5 g of the mixed finely divided sample into a dish and wash with  $H_2O$  upon the asbestos in the evaporating shell, using, if necessary, a small portion of the asbestos fiber on a stirring rod to transfer the last traces of the sample from the dish to the shell. Dry to constant weight at  $100^\circ$ , cool, wrap the glass dish loosely in smooth paper, crush into rather small fragments between the fingers, and carefully transfer the crushed mass, including the paper, to an extraction tube or a fat extraction cartridge. If a metal dish is used, cut it into small pieces and place in the extraction tube. Extract with anhydrous ether or petroleum ether (b.p.  $45-60^\circ$  and without weighable residue) in a continuous extraction apparatus for at least 25 hours. In most cases it is advisable to remove the substance from the extractor after the first 12 hours, grind with sand to a fine powder, and re-extract for the remaining 13 hours. Transfer the extract to a weighed flask, evaporate the solvent, and dry to constant weight at  $100^\circ$ .

73

*II. Roese-Gottlieb Method—Tentative*

Introduce 4 g of the material, or a quantity of a uniform soln equivalent to this weight of the dry substance, into a Rührig tube or a similar apparatus; make up to a volume of 10 cc with  $H_2O$ , add 1.25 cc of  $NH_4OH$ , and mix thoroly. Add 10 cc of 95% alcohol and mix; then add 25 cc of ether and shake vigorously for half a min.; and finally add 25 cc of petroleum ether (b.p. below  $60^\circ$ ) and shake again for half a min. Allow to stand for 20 min. or until the separation of the liquids is complete. Draw off as much as possible of the ether-fat soln (usually 0.5–0.8 cc will be left) into a weighed flask thru a small, rapid filter. Weigh the flask with a similar one as a counterpoise. Again extract the liquid remaining in the tube, this time with 15 cc each of ether and petroleum ether; shake vigorously half a minute with each solvent and allow to settle. Proceed as above, washing the tip of the spigot and the filter with a few cc of a mixture of equal parts of the two ethers (previously mixed and freed from deposited  $H_2O$ ). For a greater degree of accuracy the extraction must be repeated. If the previous ether-fat solns have been drawn off closely, this third extraction usually yields not more than about 1 mg of fat, or about 0.02% on a 4 g charge. Evaporate the ether slowly on a steam bath and then dry the fat in a boiling water oven until the loss in weight ceases. Test the purity of the fat by dissolving in a little petroleum ether. Should a residue remain, wash the fat out completely with petroleum ether, dry the residue, weigh, and deduct the weight.

74

## PARAFFIN—TENTATIVE

Add to the ether extract in the flask, as obtained under 72 or 73, 10 cc of 95% alcohol and 2 cc of  $NaOH$  soln (1+1); connect the flask with a reflux condenser; and heat for an hour on a water bath, or until saponification is complete. Remove the condenser and allow the flask to remain on the bath until the alcohol is evaporated and the residue is dry. Dissolve the residue as completely as possible in about 40 cc of  $H_2O$  and heat on the bath, shaking frequently. Wash into a separatory funnel, cool, and extract with 4 successive portions of petroleum ether, collecting the extracts in a weighed flask or capsule. Evaporate the petroleum ether and dry to constant weight at  $100^\circ$ . Any phytosterol or cholesterol present in the fat would be ex-

tracted with the paraffin, but the quantity is so insignificant that it may generally be disregarded.

**75 ALCOHOL IN SIRUPS USED IN CONFECTIONERY ("BRANDY DROPS")—OFFICIAL**

Collect in a beaker the sirup from a sufficient number of pieces to yield 30–50 g, strain into a weighed beaker, and weigh. Introduce the sirup into a 250–300 cc distillation flask, dilute with half its volume of  $H_2O$ , attach the flask to a vertical condenser, and distil almost 50 cc, or as much of the liquid as possible without causing charring. Foaming may be prevented by adding to the contents of the distillation flask a little tannin, or a piece of paraffin about the size of a pea. Cool the distillate, make up to volume with  $H_2O$ , and mix well. Determine sp. gr. as directed under XIV, 3. Calculate the percentage of alcohol by weight in the candy filling, using Table 19, XLII.

**76 COLORING MATTER—TENTATIVE**

Proceed as directed under XXI.

**77 METALS—TENTATIVE**

Proceed as directed under XXIX.

**HONEY<sup>29</sup>**

**78 PREPARATION OF SAMPLE—OFFICIAL**

(a) *Liquid or strained honey*.—If the sample is free from granulation, mix thoroly by stirring or shaking before weighing portions for the analytical determination. If the honey is granulated, place the container, having the stopper loose, in a water bath and heat at a temp. not exceeding 50° with occasional stirring until the sugar crystals dissolve. Mix thoroly, cool, and weigh portions for the analytical determinations. If foreign matter, such as wax, sticks, bees, particles of comb, etc., is present, heat the sample to 40° in a water bath and strain thru cheese cloth in a hot water funnel before weighing portions for analysis.

(b) *Comb honey*.—Cut across the top of the comb, if sealed, and separate completely from the comb by straining thru a 40-mesh sieve. When portions of the comb or wax pass thru the sieve, heat the sample as in (a) and strain thru cloth. If the honey is granulated in the comb, heat until the wax is liquefied; stir, cool, and remove the wax.

**79 MOISTURE—OFFICIAL**

Proceed as directed under 3 or 4, using a weighed quantity of the sample sufficient to yield approximately 1 g of solids; adding, if necessary, a few cc of  $H_2O$  to incorporate thoroly with the sand; and drying at 70° under a pressure of not to exceed 100 mm of Hg.

**80 ASH—OFFICIAL**

Weigh 5–10 g of honey into a Pt dish, add a few drops of pure olive oil to prevent spattering, heat carefully until swelling ceases, and ignite at a temp. not above dull redness until a white ash is obtained.

**81 SOLUBLE ASH—OFFICIAL**

Proceed as directed under 12.

**82 ALKALINITY OF THE SOLUBLE ASH—OFFICIAL**

Proceed as directed under 13.

83

## DIRECT POLARIZATION—TENTATIVE

(a) *Immediate direct polarization*.—Transfer 26 g of the honey to a 100 cc flask with  $H_2O$ , add 5 cc of alumina cream, dilute to the mark with  $H_2O$  at  $20^\circ$ , filter, and polarize immediately in a 200 mm tube.

(b) *Constant direct polarization*.—Complete the mutarotation as directed under 20. If necessary to conserve the sample, the soln from the tube used in the immediate direct polarization (a) may be returned to the flask. Make the final reading at  $20^\circ$  in a 200 mm tube.

(c) *Mutarotation*.—The difference between (a) and (b) is a measure of the mutarotation.

(d) *Direct polarization at  $87^\circ$* .—Polarize the soln obtained under (b) at  $87^\circ$  in a jacketed 200 mm metal tube, preferably of silver.

84

## INVERT POLARIZATION—TENTATIVE

(a) *At  $20^\circ$* .—Invert 50 cc of the soln obtained under 83 as directed under 22(b) or (c) or 23(b) or (c), and polarize at  $20^\circ$  in a 200 mm tube.

(b) *At  $87^\circ$* .—Polarize the soln obtained under (a) at  $87^\circ$  in a 200 mm metal tube, preferably of silver.

85

## REDUCING SUGARS—OFFICIAL

Dilute 10 cc of the soln used for direct polarization, 83, to 250 cc and determine reducing sugars in 25 cc of this soln by one of the methods given, 33, 37, or 58, respectively. Calculate the result to percentage of invert sugar.

86

## SUCROSE—OFFICIAL

(a) Calculate from the data given in 83(b) and 84(a) if inversion is conducted as directed under 22(b) or (c). Use the formula given in 22(b).

(b) Proceed as directed under 28. Determine reducing sugars after inversion by diluting 10 cc of the soln obtained under 84 with a small quantity of  $H_2O$ , neutralizing with  $Na_2CO_3$ , and making up to 250 cc with  $H_2O$ . Use 50 cc of this soln, making the determination as directed in 85.

87

## LEVULOSE—TENTATIVE

Multiply the direct reading at  $87^\circ$ , 83(d), by 1.0315 and subtract the product from the constant direct polarization at  $20^\circ$ , 83(b); divide the difference by 2.3919 to obtain the grams of levulose in a normal weight of the honey. From this figure calculate the percentage of levulose in the original sample.

88

## DEXTROSE—TENTATIVE

To obtain the approximate percentage of dextrose, subtract the percentage of levulose, 87, from the percentage of invert sugar, 85.

The dextrose can be determined more accurately by multiplying the percentage of levulose, 87, by the factor 0.915, which gives its dextrose equivalent in Cu reducing power. Subtract the figure obtained from that of the reducing sugars, 85, calculated as dextrose, to obtain the percentage of dextrose in the sample. (Owing to the difference in the reducing powers of different sugars, the sum of the dextrose thus found and the levulose as obtained under 87 will be greater than the quantity of invert sugar obtained under 85.)

89

## DEXTRIN (APPROXIMATE)—TENTATIVE

Using not more than 4 cc of  $H_2O$ , transfer 8 g of the sample (4 g in the case of dark colored honey dew honey) to a 100 cc flask by allowing the sample to drain from the weighing dish into the flask and then dissolving the residue in 2 cc of  $H_2O$ . After adding this soln to the contents of the flask, rinse the weighing dish with two 1 cc portions of  $H_2O$ , adding a few cc of absolute alcohol each time before decanting. Fill the flask to the mark with absolute alcohol, shaking constantly. Set the flask aside until the dextrin has collected on the sides and bottom and the liquid is clear. Decant the clear liquid thru a filter paper and wash the residue in the flask with 10 cc of 95% alcohol, pouring the washings thru the same filter. Dissolve the dextrin in the flask with boiling  $H_2O$  and filter thru the filter paper already used, receiving the filtrate in a weighed dish prepared as directed under 4. Rinse the flask and wash the filter a number of times with small portions of hot  $H_2O$ , evaporate on a water bath, and dry to constant weight at  $70^\circ$  under a pressure of not to exceed 100 mm of Hg.

After determining the weight of the alcohol precipitate, dissolve the latter in  $H_2O$  and make up to definite volume, using 50 cc of  $H_2O$  for each 0.5 g of precipitate or part thereof.

Determine reducing sugars in the soln both before and after inversion as directed under 30, expressing the results as invert sugar. Calculate sucrose from the results thus obtained and subtract the sum of the reducing sugars before inversion and sucrose from the weight of the total alcohol precipitate to obtain the weight of the dextrin.

90

## FREE ACID—OFFICIAL

Dissolve 10 g of the honey in  $H_2O$  and titrate with 0.1 N NaOH soln, using phenolphthalein indicator. Express the results in terms of cc of 0.1 N NaOH required to neutralize 100 g of the sample.

## COMMERCIAL GLUCOSE

91

*Qualitative Test—Tentative*

Dilute the honey with  $H_2O$  in the proportion of 1 to 1 and add a few cc of I soln (1 g of I, 3 g of KI, 50 cc of  $H_2O$ ). In the presence of commercial glucose the soln turns red or violet, the depth and character of the color depending upon the quality and nature of the glucose used. A blank test with a pure honey of about the same color should be made in order to secure an accurate color comparison. Should the honey be dark and the percentage of glucose very small, precipitate the dextrin that may be present by adding several volumes of 95% alcohol. Allow to stand until the precipitate settles (do not filter), decant the liquid, dissolve the residue of dextrins in hot  $H_2O$ , cool, and apply the above test to this soln. A negative result is not proof of the absence of commercial glucose, as some glucose, especially of high conversion, does not give any reaction with I.<sup>30</sup>

92

*Quantitative Method—Tentative*

An approximate determination can be made by Browne's formula as follows: Multiply the difference in the polarizations of the invert soln at  $20^\circ$  and  $87^\circ$ , 84, by 77 and divide this product by the percentage of invert sugar found in the sample after inversion. Multiply the quotient by 100 and divide the product by 26.7 to obtain the percentage of honey in the sample; 100% minus the percentage of honey gives the percentage of glucose.<sup>30</sup>

COMMERCIAL INVERT SUGAR<sup>31</sup>*Resorcinol Test<sup>32</sup>—Tentative*

93

## REAGENT

*Resorcinol soln.*—Dissolve 1 g of resublimed resorcinol in 100 cc of HCl (sp. gr. 1.18-1.19).

94

## DETERMINATION

Introduce 10 cc of a 50% honey soln into a test tube and add 5 cc of ether. Shake gently and allow to stand for some time until the ether layer is clear. Transfer 2 cc of this clear ether soln to a small test tube and add a large drop of the recently prepared resorcinol soln. Shake, and note the color immediately. A cherry red color appearing at once indicates the presence of commercial invert sugar. Yellow to salmon shades have no significance.

*II. Aniline Chloride Test<sup>33</sup>—Tentative*

95

## REAGENT

*Aniline chloride soln.*—To 100 cc of C. P. aniline add 30 cc of 25% HCl.

96

## DETERMINATION

Introduce 5 g of the honey into a porcelain dish and add while stirring 2.5 cc of the recently prepared aniline reagent. In the presence of commercial invert sugar, the reagent assumes *immediately* an orange-red color turning dark red. Yellow to salmon shades have no significance.

The resorcinol test and the aniline chloride test, when negative, may not be regarded as conclusive evidence of the absence of commercial invert sugar sirup in honey.

97

## DIASTASE—TENTATIVE

Mix 1 part of honey with 2 parts of sterile H<sub>2</sub>O. Treat 10 cc of this soln with 1 cc of 1% soluble starch soln and digest at 45° for an hour. At the end of this time test the mixture with 1 cc of I soln (1 g of I, 2 g of KI, 300 cc of H<sub>2</sub>O). Treat another 10 cc portion of the honey soln, mixed with 1 cc of the soluble starch soln without heating to 45°, with the reagent and compare the colors produced. If the original honey has not been heated sufficiently to destroy the diastase, an olive-green or brown coloration will be produced in the mixture that has been heated at 45°. Heated or artificial honey becomes blue.

MAPLE PRODUCTS<sup>34</sup>

98

## PREPARATION OF SAMPLE

*(a) Maple Sirup—Official*

1. *For solids determination.*—If the sample contains no sugar crystals or suspended matter, decant sufficient of the clear sirup for use in the determination. If sugar crystals are present, redissolve them by heating. If suspended matter is present, filter the sample thru cotton wool.

2. *For other determinations.*—If sugar crystals are present, redissolve them by heating. If other sediment is present, distribute it evenly thru the sirup by shaking. Transfer approximately 100 cc of the sirup, with its suspended sediment, to a casserole or beaker, add  $\frac{1}{4}$  the volume of H<sub>2</sub>O, and evaporate over a flame. When the

temp. of the boiling sirup approaches  $104^{\circ}$ , draw a small quantity into a thin-walled pipet of about 1 cc capacity, and cool to room temp. in running  $\text{H}_2\text{O}$ . Wipe the outside of the pipet, allow the possibly diluted sirup in the point to escape, and make a refractometric measurement of the solids content of the cooled sirup. Repeat this procedure from time to time until a reading is obtained corresponding to 61.5% solids ( $n_{20} = 1.4521$ ), or to such other value as in the experience of the analyst will give a filtered sirup of 65.0% solids. Filter the sirup thru a filter which will allow the 100 cc to pass within 5 min. and adjust the filtrate to  $65.0 \pm 0.5\%$  solids (refractometric) by thoro mixing with the appropriate quantity of  $\text{H}_2\text{O}$ .

(b) *Maple Sugar and Other Solid or Semi-Solid Products—Tentative*

1. *For moisture and solids determination.*—Grind in a mortar, if necessary, and mix thoroly.

2. *For other determinations.*—Prepare a sirup by dissolving approximately 100 g of the sample in 150 cc of hot  $\text{H}_2\text{O}$ , boil until the temp. approaches  $104^{\circ}$  and complete the preparation of the resulting sirup as directed in (a) 2, commencing at "draw a small quantity into a thin-walled pipet."

MOISTURE OR SOLIDS—OFFICIAL

99

*Maple Sugar*

Proceed as directed under 2, using the sample prepared as directed under 98.

100

*Maple Sirup, Maple Cream, etc.*

Proceed as directed under 3, 4 or 7, using the sample prepared as directed under 98. In the refractometric measurement guard against dew on the prisms by circulating  $\text{H}_2\text{O}$  thru the prism jackets and correct the observations to  $20^{\circ}$  by use of Table XLII, 7, if temps. other than  $20^{\circ}$  are used.

101

ASH—OFFICIAL

Heat 5 g of the sirup prepared as directed under 98(a) (2) and 98(b) (2) in a 50–100 cc Pt dish over a low flame until completely charred. Transfer to a muffle furnace and heat at low redness (not over  $550^{\circ}$ ) until a white ash is obtained. If desired, interrupt the ashing when nearly all the C is burned, and after cooling add 0.5–1.0 cc of  $\text{H}_2\text{O}$ . Evaporate, and return the dish to the muffle. To the cooled ash add about 1 g of  $(\text{NH}_4)_2\text{CO}_3$  free from non-volatile matter, and 0.5–1.0 cc of  $\text{H}_2\text{O}$ ; evaporate to dryness, reheat in the muffle for 1–2 min., cool in a desiccator, and weigh, taking precautions to guard against, or to correct for, absorption of moisture during weighing.

102

SOLUBLE AND INSOLUBLE ASH—OFFICIAL

Proceed as directed under 12.

103

ALKALINITY OF THE SOLUBLE ASH—OFFICIAL

Proceed as directed under 13.

104

ALKALINITY OF THE INSOLUBLE ASH—OFFICIAL

Proceed as directed under 14.

105

ALKALINITY OF TOTAL ASH—OFFICIAL

Add the alkalinities of the soluble and insoluble portions (103 and 104).



## 106

## METALS—TENTATIVE

Proceed as directed under XXIX.

## POLARIZATION—OFFICIAL

## 107

*Direct Polarization*

Proceed as directed under 22(a) or 23(a).

## 108

*Invert Polarization*

(a) At 20°.—Proceed as directed under 22(b) or (c) or 23(b) or (c).

(b) At 87°.—Proceed as directed under 30.

## 109

## SUCROSE—POLARIMETRIC METHODS—OFFICIAL

Proceed as directed under 22 or 23, or calculate from the results of 107 and 108, using the appropriate formula from 22 or 23.

## SUCROSE—CHEMICAL METHODS

## 110

*By Reducing Sugars Before and After Inversion—Official*

Proceed as directed under 28.

## 111

## REDUCING SUGARS AS INVERT SUGAR—OFFICIAL

(a) *Before inversion*.—Proceed as directed under 35 or 37, using an aliquot of the soln used for direct polarization, 107, and only neutral Pb acetate for clarification.

(b) *After inversion*.—Proceed as directed under 33 or 37, using an aliquot of the soln used for the invert polarization, 108(a), and only neutral Pb acetate for clarification.

## 112

## COMMERCIAL GLUCOSE—OFFICIAL

Proceed as directed under 29 or 30.

## LEAD NUMBER

*Canadian Lead Number<sup>36</sup> (Fowler Modification)—Official*

## 113

## REAGENT

*Standard basic lead acetate soln*.—Activate litharge by heating it to 650–670° for 2.5–3 hours in a muffle. (The cooled product should be lemon color.) In a 500 cc Erlenmeyer flask provided with a return condenser boil 80 g of normal Pb acetate crystals and 40 g of the freshly activated litharge with 250 g of H<sub>2</sub>O for 45 min. Cool, filter off any residue, and dilute with recently boiled H<sub>2</sub>O to a density of 1.25 at 20°.

## 114

## DETERMINATION

Weigh the quantity of sirup containing 25 g of dry matter, transfer to a 100 cc flask, and make up to mark at 20°, or use the soln in which the conductivity value has been determined (118). Pipet 20 cc into a large test tube, add 2 cc of the standard basic Pb acetate soln, cork, and allow to stand 2 hours.

Filter with suction on a 25 cc tared Gooch, having an asbestos mat at least 3 mm thick. When nearly all the liquid has run thru, fill the crucible with cold H<sub>2</sub>O. Repeat to a total of 4 washings, taking care to prevent formation of fissures in the precipitate by keeping it covered with H<sub>2</sub>O and avoiding too great suction. Dry at 100°, weigh, and multiply the weight by 20.

*Winton Lead Number*<sup>37</sup>—Official

115

## REAGENT

*Standard basic lead acetate soln.*—To a measured volume of the reagent prepared for determination of the Canadian lead number, 113, add 4 volumes of  $H_2O$ , and filter. A blank should be run with each set of determinations.

116

## DETERMINATION OF LEAD IN THE BLANK

Transfer 25 cc of the standard basic Pb acetate to a 100 cc flask, add a few drops of glacial acetic acid, and make up to the mark with  $H_2O$ . Shake, and determine  $PbSO_4$  in 10 cc of the soln as directed under 117. The use of acetic acid is imperative in order to retain all Pb in soln when the reagent is diluted with  $H_2O$ .

117

## DETERMINATION

Transfer 25 g of the sample to a 100 cc flask by means of  $H_2O$ . Add 25 cc of the standard basic Pb acetate soln and shake. Fill to the mark, shake, and allow to stand for at least 3 hours before filtering. Pipet 10 cc of the clear filtrate into a 250 cc beaker, add 40 cc of  $H_2O$  and 1 cc of  $H_2SO_4$ , shake, and add 100 cc of 95% alcohol. Allow to stand overnight, filter on a weighed Gooch crucible, wash with 95% alcohol, dry in a water oven, and ignite in a muffle or over a Bunsen burner, applying the heat gradually at first and avoiding a reducing flame. Cool, and weigh. Subtract the weight of  $PbSO_4$  so found from the weight of  $PbSO_4$  found in the blank, 116, and multiply by the factor 27.33. The use of this factor gives the Pb number directly without the various calculations otherwise required.

CONDUCTIVITY VALUE<sup>38</sup>—OFFICIAL

118

## APPARATUS

(1) *Conductivity cell.*—Should be made of resistance glass with platinized Pt electrodes firmly fixed and adequately protected from displacement. These electrodes may be sealed into a vessel into which the soln under examination may be run and subsequently drawn off (*Zerban type*), or attached to a support so that they can be lowered into a cylinder (or a 100 cc beaker) containing the soln (dipping type). The cell must be provided with a thermometer graduated in tenths of degrees and covering the range of 20–30°, and the bulb must be placed in the immediate vicinity of the electrodes. The cell constant should be approximately 0.15.

(2) *Galvanometer or a microphone hummer (or an induction coil) and a sensitive telephone receiver.*

(3) *Suitable source of current.*—Dry or storage cells if a hummer or induction coil is used; 110 volt alternating current if a galvanometer is used.

(4) *Resistances of 10 and 100 ohms.*—Should be fixed and accurate.

(5) *Slide wire or Wheatstone bridge.*

(6) *Device for control of the temp. of the cell to within  $\pm 0.1^\circ$ .*—This may consist of a thermostat or of a vessel into which  $H_2O$  of suitable temp. may be run so as to adjust the cell contents to 25°.

119

## DETERMINATION OF THE CELL CONSTANT

Prepare solns of 0.3728 and 0.7456 g of dry KCl in  $H_2O$ , which offers a resistance of at least 25,000 ohms in the cell, and make them to the mark at 20–25° in 500 cc volumetric flasks. Fill the cell with the more dilute (0.01 M) soln, adjust to 25° ±

0.1°, measure the electrical resistance, and multiply the number of ohms by 141.2. Rinse with the stronger (0.02 *M*) soln, fill the cell with this soln, measure its resistance at 25°, and multiply by 276.1. Average the two results.

## 120

## DETERMINATION

Weigh out a quantity of sirup that contains 25 g of dry matter, transfer to a 100 cc volumetric flask with warm H<sub>2</sub>O of the same quality as that used in the determination of the cell constant, cool to 25°, make to mark, and measure the resistance in the cell at 25° ± 0.1°. Divide the cell constant by the number of ohms found.

## 121

MALIC-ACID VALUE (COWLES)<sup>28</sup>—TENTATIVE

Weigh 6.7 g of the sample into a 200 cc beaker; add 5 cc of H<sub>2</sub>O, then 2 cc of a 10% Ca acetate soln; and stir. Add, gradually and with constant stirring, 100 cc of 95% alcohol and agitate the soln until the precipitate settles, or let stand until the supernatant liquid is clear. Filter off the precipitate and wash with 75 cc of alcohol, 85% by volume. Dry the filter paper and ignite in a Pt dish. Add 10 cc of 0.1 *N* HCl, II, 19(a), and warm gently until all the lime dissolves. Cool, and titrate back with 0.1 *N* NaOH soln, using methyl orange indicator. The difference in cc divided by 10 represents the malic acid value of the sample. Previous to use, the reagents should be tested by a blank determination and any necessary corrections applied.

## SUGAR BEETS

## SUCROSE

## 122

1. Alcohol Extraction Method<sup>10</sup>—Tentative

Pass the sample (usually in the form of cossettes) thru a meat grinder fitted with a plate having  $\frac{1}{8}$ -inch perforations and mix thoroly. Weigh 26 g of the beet pulp and transfer to a 100 cc flask with about 50 cc of 90% alcohol and 3-5 cc of basic Pb acetate soln. Connect a reflux condenser to the flask and place on a boiling water bath for 10-15 min. Then pour the whole into a Soxhlet extractor, washing out the flask with fresh portions of 90% alcohol. Connect the same 100 cc flask to the extractor and fit the latter with a return condenser. Add 90% alcohol until the siphon is started and the flask is about  $\frac{3}{4}$  full. Place the flask in a covered water bath kept at a temp. that will allow the alcohol to boil freely. Continue the extraction for 1-4 hours, or until a test of the alcohol in the extractor gives no color with alpha-naphthol soln when tested as follows: Introduce into a test tube a few drops of the alcohol coming from the extractor and add 4 or 5 drops of a 20% alcoholic alpha-naphthol soln and 2 cc of H<sub>2</sub>O. Shake well, tip the tube, allow 2-5 cc of colorless H<sub>2</sub>SO<sub>4</sub> to flow down the side of the tube, then hold the tube upright. If sucrose is present, a color varying from a faint to deep violet will be noted at the junction of the two liquids. On shaking, the whole soln becomes a blue violet color. This test is suitable for this work, but it must be remembered that other substances besides sucrose give this color reaction.

Remove the flask from the water bath, transfer the contents to a 100 cc volumetric flask, cool to the standard temp., dilute to the mark with 90% alcohol, shake, and filter, keeping the funnel covered with a watch-glass. Polarize in a 200 mm tube.

Avoid evaporation and changes of temp. and use a minimum quantity of basic Pb acetate for clarification, 3 cc rather than 5 cc. By digesting the beet pulp with the alcohol before extraction, the time of extraction is greatly shortened, the pulp becomes thoroly impregnated with the alcohol, all the air is removed, and a good

extraction of the whole material is effected. If the pulp is fine and tends to clog the siphon, alcohol-washed cotton may be used as a plug in the extractor before adding the beet pulp, and a fine mesh screen may be placed over the pulp to keep the whole compact in the extractor.

## 123

*II. Hot Water Digestion Method I.<sup>11</sup>—Tentative*

Weigh 26 g of the beet pulp, prepared as directed under 122, and transfer with  $H_2O$  to a wide-mouthed flask graduated to a content of 201.0 cc, the additional 1.0 cc representing an allowance for volume of marc and of Pb precipitate; add 5-10 cc of basic Pb acetate soln and sufficient  $H_2O$  to produce a volume of about 160-170 cc, and shake. Immerse the flask in a water bath at  $80^\circ$  and rotate at intervals. At the end of 30 min. dilute to the mark with  $H_2O$  at  $80^\circ$  and continue the digestion for 10 min. longer. Remove the flask from the water bath and allow it to cool to standard temp. Add sufficient strong acetic acid<sup>12</sup> to make the soln very slightly acid (generally less than 0.5 cc) and a few drops of ether to break the foam. Apply vacuum to withdraw retained air. Dilute to the mark at  $20^\circ$ , mix thoroly, filter, and polarize in a 400 mm tube.

## 124

*III. Hot Water Digestion Method II.<sup>13</sup>—Tentative*

Use Ni-plated sheet iron vessels, 11 cm high, 6 cm body diameter, and 4 cm mouth diameter, also stoppers covered with tinfoil to fit.

Weigh 26 g of the beet pulp, prepared as directed under 122, on a watch-glass (small enough to go into the neck of the beaker) and transfer to the metal beaker; add 177 cc of dilute basic Pb acetate soln (5 parts of basic Pb acetate soln, sp. gr. 1.25, to 100 parts of  $H_2O$ ); shake, and stopper lightly. Submerge the beaker in a water bath at  $75-80^\circ$  for 30 min., shaking intermittently. When all the air has been expelled (generally after 5 min.), tighten the stopper. After 30 min., shake, cool to a standard temp., filter, add a drop of acetic acid to the filtrate, and polarize in a 400 mm tube. The reading is the percentage of sugar in the beet pulp.

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## XXXV. VEGETABLES AND VEGETABLE PRODUCTS

1

### PHYSICAL EXAMINATION—TENTATIVE

Note carefully the external appearance of the packages to detect the presence of "leakers," "swells," or "springers." In general, the ends of sound tins of canned vegetables are slightly concave. If desired, the vacuum or pressure in the can may be taken by a gage designed for that purpose.

Measure the distance from the top of the double seam to the contents of the can.

Note particularly the odor of the vegetables, the appearance of the liquor or brine—whether clear or turbid—and the condition of the seams and the inner walls of the container, especially as to blackening and corrosion; note also the general appearance, color, flavor, and size of the vegetables. In all instances the analyst should familiarize himself with the normal appearance, odor, color, flavor, and other properties of the product under examination. Make careful macroscopic or microscopic examination for worm infestation, mold, dirt, or other evidence of decomposition or filth. If analysis of the gas in a bulged can is desired, the gas may be conveniently collected by means of the Doremus gas collector<sup>1</sup> or by similar apparatus.

2

### PREPARATION OF SAMPLE—OFFICIAL

The preparation of the sample depends upon the character of the product and the determinations to be made. With samples composed of solid and liquid portions, proceed as follows: Weigh the full can, then open, make the physical measurements as directed under 1, pour the entire contents on a round sieve with a No. 8 standard screen (diameter of wire 0.84 mm and size of opening 2.38 mm).<sup>2</sup> Use a sieve 8 inches in diameter for a No. 3 or smaller can, and a sieve 12 inches in diameter for cans larger than No. 3.

Allow the can to drain 2 min., weigh either the drained solids or the free liquid direct, and reweigh the dry empty can. From the weights thus obtained determine the percentage of liquid and of solid contents. If only the solid portion is required for analysis or examination, thoroly grind the drained vegetables in a mortar or food chopper. If a composite of the solid and liquid portion is required, thoroly grind the entire contents of the can in a mortar or food chopper. In all cases, thoroly mix the portion used and preserve the balance in glass-stoppered containers. Unless the analysis is to be completed in a reasonably short time, determine the moisture in a portion of the sample prepared as above, and to prevent decomposition dry the remainder, grind, mix thoroly, and preserve in glass-stoppered containers. A second moisture determination is required in this procedure.

3

### MOISTURE—OFFICIAL

Proceed as directed under XXVII, 5.

4

### ASH—OFFICIAL

Proceed as directed under XXVII, 8.

5

### SODIUM CHLORIDE—OFFICIAL

Determine Cl as directed under XII, 35 or 37, and express the result in terms of NaCl.

6

### REDUCING SUGARS BEFORE INVERSION—OFFICIAL

Weigh 20 g of the sample into a 200 cc flask, dilute with about 100 cc of H<sub>2</sub>O, clarify with a slight excess of neutral Pb acetate soln, dilute to the mark, and filter.

Remove the excess of Pb with anhydrous  $\text{Na}_2\text{SO}_4$  or with dry K oxalate. Filter, and determine reducing sugars as directed under XXXIV, 37. Express the result as percentage of invert sugar.

7 REDUCING SUGARS AFTER INVERSION—OFFICIAL

Transfer 50 cc of the filtrate obtained under 6 to a 100 cc flask, add 5 cc of HCl, and let stand overnight, as directed under XXXIV, 23 (c). Nearly neutralize with NaOH soln, cool, dilute to the mark, and determine reducing sugars in an aliquot as directed under XXXIV, 37. Express the result as percentage of invert sugar.

8 SUCROSE—OFFICIAL

Proceed as directed under XXXIV, 28.

9 TOTAL ACIDS—OFFICIAL

Proceed as directed under XXVI, 24, using 5 g of the sample. Express the result as the number of cc of normal alkali required to neutralize 100 g of sample.

10 VOLATILE ACIDS—OFFICIAL

Proceed as directed under XV, 24. Express the result as percentage of acetic acid. 1 cc of 0.1 N alkali = 0.0060 g of acetic acid.

11 PRESERVATIVES AND ARTIFICIAL SWEETENERS

Proceed as directed under XXXII.

12 COLORING MATTERS

Proceed as directed under XXI.

13 METALS

Proceed as directed under XXIX.

TOMATO PRODUCTS<sup>1</sup>

(Tomato catsup, pulp, purée, sauce, and paste.)

14 PREPARATION OF SAMPLE—OFFICIAL

Shake the unopened container thoroly to incorporate any sediment. Transfer the entire contents to a large glass or porcelain dish and mix thoroly, continuing the stirring for at least 1 min. Transfer the well-mixed sample to a glass-stoppered container and shake or stir thoroly each time before removing portions for analysis.

15 SPECIFIC GRAVITY—TENTATIVE

Determine the sp. gr. at 20°/20°, using a National Canners Association sp. gr. bottle, Fig. 44. Clean and calibrate the bottle at 20° as directed under XIV, 3, but since the bottle is not provided with a cap, strike off excess  $\text{H}_2\text{O}$  with a straight edge, wipe the bottle dry, and weigh immediately. Cool the sample to 16–18°, fill the flask with the pulp, and place it in a centrifuge with a suitable counterpoise in the other receptacle. Whirl for 1 min. at a speed of about 1000 r.p.m. Add sufficient pulp to fill the flask to the top and whirl the centrifuge again. Remove flask and take the temp. of the pulp, inserting the thermometer so that no air is introduced. When the temp. is just 20°, remove thermometer, add sufficient pulp at the same temp. to have

the flask slightly over-full, and strike off even with a straight edge. Clean the outside of the flask and weigh at once to the nearest 0.01 g. Sp. gr. = weight of pulp in the flask  $\div$  the weight of  $H_2O$  at  $20^\circ$  that the flask holds.

#### 16 TOTAL SOLIDS—TENTATIVE

Weigh a portion of the sample into a flat-bottomed dish of such size that the dry residue will not exceed 12 mg per sq. cm of drying surface. Distribute evenly in a thin layer over the bottom of the dish. Place in a vacuum oven at  $70^\circ$ , with release cock left partly open so that the degree of vacuum does not exceed 450 mm of Hg and the moisture evolved is carried off rapidly. After reaching apparent dryness (approximately 1 hour), nearly close the release cock and dry at  $70^\circ$  for 4 hours at a pressure not to exceed 100 mm.

#### 17 INSOLUBLE SOLIDS—TENTATIVE

Wash 20 g of the sample repeatedly with hot  $H_2O$ , centrifuging after each addition of  $H_2O$  and pouring the clear, supernatant liquid thru a weighed paper filter on a Büchner funnel. The filter used is one of two such papers dried 2 hours at  $100^\circ$  and weighed in a covered dish. The second paper is used, if necessary, when the first paper becomes clogged. After 4 or 5 washings transfer the remaining insoluble matter to the filter, dry in the covered dish for 2 hours at  $100^\circ$ , cool in a desiccator, and weigh.

#### 18 SOLUBLE SOLIDS—TENTATIVE

To obtain the percentage of soluble solids, subtract the percentage of insoluble solids from the percentage of total solids.

#### 19 SAND—TENTATIVE

Weigh 100 g of the well-mixed sample into a 2-3 liter beaker, nearly fill the beaker with  $H_2O$ , and mix the contents thoroly. Allow to stand 5 min. and decant the supernatant liquid into a second beaker. Refill the first beaker with  $H_2O$  and again mix the contents. After 5 min. more decant the second beaker into a third and the first into the second; refill and again mix the first. Continue this operation, decanting from the third beaker into the sink until the lighter material is washed from the sample. Then collect the sand from the 3 beakers on a weighed Gooch crucible, dry, ignite, and weigh.

Under "Sand" only the figure obtained by the above method should be reported. The results obtained by the determination of ash insoluble in HCl are not applicable to the determination of sand, as the sand is so unevenly distributed that reliable results can be obtained only by taking a larger sample than is possible in the determination of ash.

#### 20 ASH—OFFICIAL

Evaporate 10 g of the sample to dryness on a water bath and ignite as directed under XXVII, 8.

#### 21 ALKALINITY OF THE ASH—OFFICIAL

Proceed as directed under XXVI, 10. Express the result as the number of cc of normal acid required to neutralize the ash from 100 g of the sample.



FIG. 44—BOTTLE USED IN DETERMINATION OF SPECIFIC GRAVITY OF TOMATO PRODUCTS



**22 SODIUM CHLORIDE—OFFICIAL**

Determine Cl as directed under **XII, 35 or 37**, using a  $\text{HNO}_3$  soln of the ash (cf. **XII, 33 and 35**). Calculate and report the result as percentage of NaCl.

**23 SUGARS**

Proceed as directed in **6, 7, and 8**.

**24 TOTAL ACIDS—OFFICIAL**

Proceed as directed under **XXVI, 24**, using 5 g of the sample. Express the result as percentage of anhydrous citric acid. 1 cc of 0.1 *N* alkali = 0.0064 g of anhydrous citric acid.

**25 VOLATILE ACIDITY—OFFICIAL**

Proceed as directed under **XV, 24**, using 25 g of the sample, increasing the quantity of  $\text{H}_2\text{O}$  used for the distillation, and collecting a correspondingly larger quantity of distillate. Express the result as percentage of acetic acid. 1 cc of 0.1 *N* alkali = 0.0060 g of acetic acid.

**26 FIXED ACIDITY—OFFICIAL**

Multiply the percentage of volatile acids, **25**, by 1.067 and subtract the product from the percentage of total acids, **24**, to obtain the percentage of fixed acids as citric acid.

**MICROANALYSIS OF TOMATO PULP, PURÉE, SAUCE, PASTE<sup>5</sup>—OFFICIAL****27 APPARATUS**

(a) *Compound microscope*.—Equipped with good objectives and oculars, giving magnifications of approximately 90, 180, and 500 diameters. For convenience of use the lenses should be adjusted so as to be parfocal. A mechanical stage is highly desirable. It is essential that the combination giving the low magnification be capable of adjustment to give an area of the field of view of 1.5 sq. mm (a circle whose diameter is 1.382 mm). With the higher powers the working distances must be ample to allow the free use of the blood-counting cell.

(b) *Drop-in cross-ruled disk*.<sup>6</sup>—For estimating lengths of mold filaments, an ocular drop-in disk cross-ruled in sixths of the ocular diaphragm opening is desirable.

(c) *Blood counting cell*.—Ruled in the Thoma or the old Neubauer system of rulings. The so-called "improved" system of Neubauer is not suitable for this purpose.

(d) *Howard mold-counting cell*.<sup>7</sup>—Constructed like a blood-counting cell but with unruled central disk about 19 mm in diameter.

**28 MOLDS**

In making mold counts of tomato juice, catsup, and purée, use the product as is, but with tomato pastes mix first with  $\text{H}_2\text{O}$  so that the tomato solids will give a sp. gr. of 1.035. If the paste contains salt or other substance that influences the sp. gr. materially, take this into consideration in making the dilution.

Clean the special Howard cell so that Newton's rings are produced between the slide and the cover-glass. Remove the cover and place a small drop of the well-mixed sample upon the central disk; using a knife blade or scalpel, spread the drop evenly over the disk, and cover with the glass so as to give an even spread to the material.

It is of the utmost importance that the drop be taken from a thoroly mixed sample

and spread evenly over the slide disk. Otherwise, when the cover slip is put in place the insoluble material, and consequently the molds, may be more abundant at the center of the mount. Avoid using a drop that is much greater than is sufficient to fill the space between the center disk and cover slip. Discard any mount showing uneven distribution, absence of Newton's rings, or liquid that has been drawn across the mount and under the cover glass.

Place the slide under the microscope and examine with such adjustment that each field of view covers 1.5 sq. mm. This area, which is of vital importance, may frequently be obtained by adjusting the draw-tube in such a way that the diameter of the field becomes 1.382 mm. Where such adjustment by means of the draw-tube is not possible, it is sometimes necessary to have a mechanic make an accessory drop-in ocular diaphragm with the aperture accurately cut to the necessary size. The diameter of the area of the field of view can be determined by use of a stage micrometer, or by employing the rulings on the blood-counting cell. In order to use the latter method it is necessary to bear in mind that a square whose diagonal is 1.382 mm has sides of approximately 0.977 mm. Hence the mm scale on the blood-counting cell can be used by such adjustment that the circle of the field of view cuts off the necessary amount from each corner of the mm-ruled square. When the instrument is properly adjusted, the quantity of liquid examined per field is 0.15 cmm (0.00015 cc).

Examine at least 25 fields from each of two or more mounts taken in such manner as to be representative of all sections of the mount. Observe each field, noting the presence or absence of mold filaments and recording the result as positive or as negative, as the case may be. No field should be considered positive unless the aggregate length of not more than three of the filaments present exceeds approximately  $\frac{1}{4}$  of the diameter of the field. Calculate the proportion of positive fields from the results of the examination of all the observed fields and report as percentage of fields containing mold filaments.

## 29

## YEASTS AND SPORES

Fill a graduated cylinder with  $H_2O$  to the 20 cc mark and add the sample till the level of the mixture reaches the 30 cc mark. Close the graduate, or pour the contents into an Erlenmeyer flask, and shake vigorously for 15-20 seconds. To assure thoroughness the mixture should not fill more than  $\frac{2}{3}$  of the container in which the shaking is performed. For tomato sauce or pastes, or products running high in the number of organisms or of heavy consistency, use 80 cc of  $H_2O$  with 10 cc or 10 g of the sample. In the case of exceptionally thick or dry pastes, it may be necessary to make an even greater dilution.

Pour the mixture into a beaker. Thoroughly clean the counting cell so as to give good Newton's rings. Stir thoroughly the contents of the beaker with a scalpel or knife blade and after allowing to stand 3-5 seconds remove a small drop, place it upon the central disk of the blood-counting cell, and cover immediately with the cover-glass, observing precautions to secure production of Newton's rings and avoiding the overflowing of the liquid so as to run in between the cover and supporting surfaces of the slide. Allow the slide to stand not less than 10 min. before beginning to make the count. It is customary to make the count with a magnification of about 180 to 220 diameters.

Count the number of yeasts and mold spores on  $\frac{1}{4}$  of the ruled squares on the disk (this amounts to counting the number in 8 of the blocks, each of which contains 25 of the small ruled squares). The total number thus obtained equals the number of organisms in 1/60 cmm (1/60,000 cc) if a dilution of 1 part of the sample with 2

parts of  $H_2O$  is used. If a dilution of 1 part of the sample with 8 parts of  $H_2O$  is used, the number must be multiplied by 3. In making the counts, the analyst should avoid counting twice the organisms that rest on a boundary line between two adjacent squares.

## 30

## BACTERIA

Determine the number of rod-shaped bacteria from the mounted sample used in 28, but before examination allow the sample to stand not less than 15 min. after mounting. Use a magnification of about 500 diameters.

Count and record the number of bacteria having a length greater than  $1\frac{1}{2}$  times their width in an area including 5 of the small sized squares. Count the number in 5 such areas, preferably 1 from near each corner of the ruled portion of the slide and 1 from near the center. Determine the total number of rod-shaped bacteria in the 5 areas and multiply by 480,000. This gives the number of this type of bacteria per cc. If a dilution of 1 part of the sample with 8 parts of  $H_2O$ , instead of 1 part of the sample with 2 parts of the  $H_2O$ , is used in making up the sample, then the total count obtained in the 5 areas must be multiplied by 1,440,000; The bacteria sometimes exhibit a slight motion and thus may present momentarily an end instead of a side view. For this reason it is necessary to keep them under observation long enough to establish their true character. Thus far it has proved impracticable to count the micrococci present as they are likely to be confused with other bodies frequently present in such products.

## 31 FIELD CORN IN CANNED MIXTURES OF FIELD AND SWEET CORN—TENTATIVE

Empty the contents of a No. 2 can, or the representative equivalent portion of a larger can, into a large beaker and remove the liquor and debris from fragments of kernels by flotation with cold  $H_2O$ . Place upon a flat plate all kernels to which the outer seed coat is still attached, mix thoroly, and quarter to about 400 pieces. Harden the selected pieces in 95% alcohol and quarter again to obtain about 100 fragments. Cut each fragment thru with a section razor or knife and avoid contamination of the fragments with dextrin by washing and drying the instrument after each cut. With a dissecting needle remove a portion about  $\frac{1}{8}$  in. in diameter from the uncontaminated interior of each kernel and place the pieces in separate depressions of a white spot plate. Cover each piece with freshly prepared I stain (0.2 g of I, 1.5 g of KI in 100 cc of  $H_2O$ ) and allow to stand 10 min. A brown cloud will disseminate from the portions of sweet corn due to dextrin, while the soln surrounding the field corn will remain clear and the portion will be blue black and sharply outlined. Crush the field corn portions to insure absence of dextrin and count those found to contain none. Use care in interpreting the results because kernels of immature sweet corn do not contain enough dextrin to produce the dense brown coloration characteristic of more mature sweet corn. In case of doubt, report as field corn only those kernels having a firm texture and showing no brown coloration with I soln on 2 confirmatory tests. Calculate the percentage of field corn from the total number of kernels examined.

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### XXXVI. VITAMINS\*

\* See note at bottom of p. xvii.

## XXXVII. WATERS, BRINE, AND SALT

### WATERS

#### POTABLE WATER

##### TURBIDITY—OFFICIAL

1

##### REAGENTS

(a) *Standard turbidity soln.*.—Mix 1 g of elutriated fullers' earth, previously dried and sifted thru a 200-mesh sieve, with  $H_2O$  and dilute to 1 liter. If the fullers' earth is of good quality and the proper degree of fineness, this stock mixture has a turbidity of 1000. Check the stock soln with a turbidimeter equipped with either candle or electric light.

(b) *Turbidity standards.*.—Prepared by dilution of (a).

2

##### DETERMINATION

If the turbidity is less than 100, which prohibits the use of the turbidimeter, determine by direct comparison with turbidity standards contained in bottles of clear white glass.

##### COLOR—OFFICIAL

3

##### REAGENTS

(a) *Standard color soln.*.—Dissolve 1.245 g of potassium chloroplatinate ( $PtCl_4 \cdot 2KCl$ ) containing 0.5 g of Pt, and 1 g of crystallized  $CoCl_2 \cdot 6H_2O$  containing 0.25 g of Co, in a small quantity of  $H_2O$ ; add 100 cc of  $HCl$  and dilute to 1 liter with  $H_2O$ . This stock mixture has a color of 500.

(b) *Color standards.*.—Prepared by dilution of (a).

4

##### DETERMINATION

Compare the color of the sample, freed from suspended matter, with the standards in tubes of clear white glass.

5

##### ODOR—OFFICIAL

Shake the vessel containing the sample and note the odor. Heat a portion of the sample to incipient boiling and note the odor.

6

##### TOTAL SOLIDS—OFFICIAL

Thoroughly shake the vessel containing the sample and pipet 100 cc of the unfiltered  $H_2O$  into a weighed Pt dish. If the sample contains much suspended matter, shake, pour rapidly into a 100 cc measuring cylinder, and transfer without delay to a weighed Pt dish. Evaporate to dryness and heat to constant weight at  $100^\circ$ . In the case of highly mineralized waters it is advisable to weigh again after drying at  $180^\circ$ .

7

##### SOLIDS IN SOLUTION—OFFICIAL

Allow the sample to stand until all sediment has settled and filter if necessary to secure a perfectly clear liquid. Occasionally a clear filtrate can be obtained only by the use of alumina cream, but this should be avoided if possible. Evaporate 100–250 cc to dryness in a weighed Pt dish. Heat to constant weight at  $100^\circ$ . In the case of highly mineralized waters it is advisable to weigh again after drying at  $180^\circ$ .

8

## SUSPENDED MATTER—OFFICIAL

(1) Subtract the value for solids in soln, 7, from the value for total solids, 6; or,  
(2) filter a suitable measured volume of the sample thru a dry weighed Gooch crucible containing an asbestos mat, dry crucible and contents at 100°, cool, and weigh.

9

## IGNITED RESIDUE—OFFICIAL

Ignite the residue from 6 at a low red heat until the ash is white or nearly so. Note any odor or change in color produced during ignition. Record the weight of the ignited residue and calculate the loss on ignition.

## NITROGEN IN THE FORM OF FREE AND ALBUMINOID AMMONIA

*Method I.—Official*

(For waters that do not contain hydrogen sulfide.)

10

## REAGENTS

(a) *Nessler's reagent*.—Dissolve 143 g of NaOH in 950 cc of H<sub>2</sub>O and filter thru asbestos. Add 50 g of red HgI<sub>2</sub> to the filtrate and dilute with H<sub>2</sub>O to 1 liter. Mix thoroly, allow to settle, and use the supernatant liquid.

(b) *Alkaline potassium permanganate soln*.—Dissolve 143 g of NaOH and 8 g of KMnO<sub>4</sub> in H<sub>2</sub>O and dilute to 1 liter.

11

## DETERMINATION

Connect a flask of about 1000 cc capacity with an upright bulb condenser by means of a rather large glass tube and a soft rubber stopper or a recently extracted cork stopper. Place in the flask 5 cc of a saturated soln of Na<sub>2</sub>CO<sub>3</sub> and 500 cc of NH<sub>3</sub>-free H<sub>2</sub>O. Distil into 50 cc Nessler tubes until no further traces of NH<sub>3</sub> are indicated on the addition of 2 cc of the Nessler reagent to 50 cc of the distillate. Continue the distillation until the volume of the soln in the flask has been reduced to about 200 cc. Cool slightly, add 500 cc of the sample under examination, and distil, at the rate of about 1 tubeful in 10 min., into 50 cc Nessler tubes until NH<sub>3</sub> ceases to be given off (4 or 5 tubes are usually sufficient). Add 2 cc of the Nessler reagent to each tube and let stand 10 min. Freshly prepare in a similar manner other nesslerized tubes containing known quantities of the standard NH<sub>4</sub>Cl soln, made up to 50 cc with NH<sub>3</sub>-free H<sub>2</sub>O, and compare the nesslerized distillates with these. Report as mg per liter of N in the form of free NH<sub>3</sub>. Cool the flask and add 50 cc of the alkaline permanganate recently boiled. Distil, at the rate of 1 tubeful in 10 min., into 50 cc Nessler tubes until NH<sub>3</sub> ceases to come off. Nesslerize and compare as in the determination of free NH<sub>3</sub>. Report as mg per liter of N in the form of albuminoid NH<sub>3</sub>.

*Method II.—Official*

(For waters containing sulfur<sup>1</sup>.)

12

## REAGENTS

(a) *Sulfuric acid soln*.—Dilute 7 cc of H<sub>2</sub>SO<sub>4</sub> (free from NH<sub>3</sub>-salts) to 500 cc.

(b) *Sodium carbonate soln*.—Dissolve 66 g of anhydrous Na<sub>2</sub>CO<sub>3</sub> or 179 g of Na<sub>2</sub>CO<sub>3</sub>·10 H<sub>2</sub>O in H<sub>2</sub>O and dilute to 250 cc.

The other reagents and solns used are described under 10.

13

## DETERMINATION

Place 500 cc of the sample in a beaker or casserole, add 30 cc excess of the H<sub>2</sub>SO<sub>4</sub> soln (a) and boil carefully until free from sulfide (about 20 min.). Add about 300 cc

of  $\text{H}_2\text{O}$  and 8 cc of the  $\text{Na}_2\text{CO}_3$  soln to a distilling flask connected as described under 11 and distil until free from  $\text{NH}_3$ . Cool, add the cooled sample, which has been freed from sulfide, and proceed with the distillation, addition of alkaline permanganate soln, etc., as directed under 11.

#### NITROGEN IN THE FORM OF NITRITE—OFFICIAL

14

##### REAGENTS

(a) *Sulfanilic acid soln.*—Dissolve 1 g of sulfanilic acid in hot  $\text{H}_2\text{O}$ , cool, and dilute to 100 cc.

(b) *Alpha-naphthylamine hydrochloride soln.*—Boil 0.5 g of the salt with 100 cc of  $\text{H}_2\text{O}$ , kept at constant volume, for 10 min.

(c) *Standard nitrite soln.*—Dissolve 1.1 g of  $\text{AgNO}_2$  in  $\text{NO}_2$ -free  $\text{H}_2\text{O}$ , precipitate the Ag with  $\text{NaCl}$  soln, dilute to 1 liter, mix, and allow to settle. Dilute 100 cc to 1 liter and then 10 cc of this soln to 1 liter, using in each case  $\text{NO}_2$ -free  $\text{H}_2\text{O}$ . 1 cc of the last soln = 0.0001 mg of N as  $\text{NO}_2$ .

15

##### DETERMINATION

Place 100 cc of the sample in a 100 cc Nessler tube and treat with 1 or 2 drops of  $\text{HCl}$ . Add 1 cc of the sulfanilic acid, 1 cc of the alpha-naphthylamine hydrochloride soln, and thoroly mix. Set aside for 30 min. with other Nessler tubes containing known quantities of the standard  $\text{NO}_2$  soln made up to 100 cc with  $\text{NO}_2$ -free  $\text{H}_2\text{O}$  and treated with  $\text{HCl}$ , sulfanilic acid, and alpha-naphthylamine hydrochloride soln in the same manner as the sample. Determine the quantity of  $\text{NO}_2$  by comparing the depth of pink color in the known and unknown solns. Record as N in the form of  $\text{NO}_2$ .

#### NITROGEN IN THE FORM OF NITRATE.

##### 1. Phenoldisulfonic Acid Method—Official

(For water of low chlorine content.)

16

##### REAGENTS

(a) *Phenoldisulfonic acid soln.*—Dissolve 25 g of pure white phenol in 150 cc of  $\text{H}_2\text{SO}_4$ , add 75 cc of fuming  $\text{H}_2\text{SO}_4$  (13–15%  $\text{SO}_3$ ), and heat at  $100^\circ$  for 2 hours.

(b) *Standard nitrate soln.*—Dissolve 0.607 g of pure  $\text{NaNO}_3$  in 1 liter of  $\text{NO}_2$ -free  $\text{H}_2\text{O}$ . Evaporate 50 cc of this soln to dryness in a porcelain dish; when cool, treat with 2 cc of the phenoldisulfonic acid soln, rubbing with a glass rod to insure intimate contact; and dilute to 500 cc. 1 cc = 0.01 mg of N as  $\text{NO}_3$ . (This soln is permanent.) Prepare standards for comparison by adding  $\text{NH}_4\text{OH}$  to measured volumes of the standard soln in 100 cc Nessler tubes.

(c) *Standard silver sulfate soln.*—Dissolve 1.397 g of  $\text{Ag}_2\text{SO}_4$ , free from  $\text{NO}_3$ , in 1 liter of  $\text{H}_2\text{O}$ . 1 cc = 1 mg of Cl.

17

##### DETERMINATION

To 100 cc of the sample, or a quantity that contains 0.05 mg or less of N as  $\text{NO}_3$ , add the standard  $\text{Ag}_2\text{SO}_4$  soln, precipitating all but about 0.5 mg of the Cl. Heat to boiling and allow to settle, or add a little alumina cream, filter, and wash with small quantities of hot  $\text{H}_2\text{O}$ . Evaporate the filtrate to dryness in a porcelain dish on a steam bath; when cool, treat with 2 cc of the phenoldisulfonic acid soln as directed under 16(b). Dilute with  $\text{H}_2\text{O}$  and add slowly  $\text{NH}_4\text{OH}$  until the maximum color is

developed. Filter if necessary, transfer to a colorimetric cylinder, and compare with the standards in the usual manner. Record as N in the form of  $\text{NO}_3$ .

## II. Reduction Method—Official

(For water of high chlorine content.)

### 18

#### REAGENTS

(a) *Aluminum foil*.—Should be the purest obtainable. Cut into strips about 10 cm long, weighing about 0.5 g.

(b) *Sodium hydroxide soln.*—Dissolve 250 g of the purest NaOH obtainable in 1250 cc of  $\text{H}_2\text{O}$ . Add 2 or 3 strips of the Al foil and let stand about 12 hours. Concentrate the soln to 1 liter by boiling.

### 19

#### DETERMINATION

To 100 cc of the sample, or a quantity that contains 0.1 mg or less of N as  $\text{NO}_3$ , in a 300 cc casserole, add 2 cc of the NaOH soln and concentrate by boiling to about  $\frac{1}{3}$  the original volume. Transfer to a 100 cc test tube, using N-free  $\text{H}_2\text{O}$ , and dilute, if necessary, to a volume of about 75 cc. Prepare a blank (preferably several blanks, since the N impurity in Al is often distributed unevenly) by placing about 75 cc of N-free  $\text{H}_2\text{O}$  and 2 cc of the NaOH soln in a 100 cc test tube. Place a strip of the Al foil in each tube. Close the ends of the test tubes with rubber stoppers connected by means of bent glass tubes with other test tubes containing about 50 cc of slightly acidified  $\text{NH}_3$ -free  $\text{H}_2\text{O}$ . (These latter tubes serve as traps to prevent the escape of  $\text{NH}_3$  and at the same time permit the free evolution of  $\text{H}_2$ .) Allow the sample and blank to stand at room temp. for 12 hours or until reduction is complete. Nesslerize the traps. If high in  $\text{NH}_3$ , indicating frothing over of the sample, discard the determination. Disregard the traps if they contain only 0.01–0.02 mg of N as  $\text{NH}_3$  each. Transfer the sample and blank to distillation flasks, using 250 cc of  $\text{NH}_3$ -free  $\text{H}_2\text{O}$  for each; distil, nesslerize, and compare with standards as in the determination of free  $\text{NH}_3$ , 11. Subtract the quantity of N found in the blank from that found in the sample. Calculate to mg per liter of N in the form of  $\text{NO}_3$ .

#### CHLORIDE—OFFICIAL

### 20

#### REAGENTS

(a) *Potassium chromate indicator*.—Dissolve 5 g of  $\text{K}_2\text{CrO}_4$  in  $\text{H}_2\text{O}$ , add a soln of  $\text{AgNO}_3$  until a slight permanent red precipitate is produced, filter, and dilute to 100 cc.

(b) *Standard silver nitrate soln.*—Dissolve 4.791 g of  $\text{AgNO}_3$  in  $\text{H}_2\text{O}$  and dilute to 1 liter. 1 cc = 1 mg of Cl. Check by titration against a standardized soln of NaCl.

### 21

#### DETERMINATION

To 100 cc of the sample add a few drops of phenolphthalein indicator. If a pink color appears, titrate the  $\text{CO}_3$  thus indicated to  $\text{HCO}_3$  with 0.05 N  $\text{H}_2\text{SO}_4$ . If the sample is acid to methyl orange, add 0.05 N  $\text{Na}_2\text{CO}_3$  to neutralize the acidity. Add 1 cc of the  $\text{K}_2\text{CrO}_4$  indicator and titrate with the standard  $\text{AgNO}_3$  soln. Correct for the quantity of  $\text{AgNO}_3$  soln necessary to give, in 100 cc of Cl-free  $\text{H}_2\text{O}$  with 1 cc of the chromate, the shade obtained at the end of the titration of the sample. (If iodides and bromides are found in interfering quantities, make the equivalent correction.)



If chlorides are present in very small quantities, concentrate 500 or 1000 cc in a porcelain dish to 100 cc, rub down the sides of the dish carefully, add 1 cc of the  $K_2CrO_4$  indicator, and titrate with the standard  $AgNO_3$  soln. If sufficient chlorides are present in 100 cc of the  $H_2O$  to consume more than 25 cc of the standard  $AgNO_3$  soln, determine by precipitation and weigh the  $AgCl$  as directed under XII, 35.

## FLUORIDES—TENTATIVE

## 22

## REAGENTS

(a) *Standard fluoride soln.*—1 cc = 0.02 mg of F. Prepare by diluting a stock soln containing 1 g of F per liter. NaF may be weighed out directly (2.22 g to 1 liter).

(b) *Titanium soln.*—To 2 cc of 20%  $TiCl_3$  add 40 cc of  $HNO_3$  (1+1) and make to 1 liter with  $H_2O$ .

(c) *Hydrogen peroxide soln.*—Dilute 10 cc of 30%  $H_2O_2$  to 100 cc with  $H_2O$ .

(d) *Copper nitrate soln.*—Dissolve 5 g of  $Cu(NO_3)_2$  in 100 cc of  $H_2O$ .

Test the  $H_2SO_4$  for presence of F. (A straight Willard and Winter distillation<sup>5</sup> will suffice.) Collect 175 cc distillate and make to 200 cc volume.

## 23

## APPARATUS

(a) *Claissen flask.*—Capacity 125 cc.

(b) *Nessler tubes.*—At least 7 long-form 50 cc tubes, and more if possible. Tubes with fused bottoms of optical glass are desirable but not absolutely necessary. (Fisher Scientific Company's "Double Plane" tubes are recommended.) Match the tubes for length and test for optical similarity by filling to the mark with a soln corresponding to the "0.04" standard described later. Reject all tubes showing detectable differences in shade or intensity.

(c) *pH comparator.*—Use any instrument that will show that the pH of a colored soln equals  $1.50 \pm 0.02$  pH. A special set made by the LaMotte Company has standards of 1.40, 1.45, 1.50, 1.55, and 1.60 pH units with metacresol purple indicator.

## 24

## PREPARATION OF SAMPLE

Make a preliminary examination to ascertain the approximate quantity of F present by comparing in Nessler tubes 10 cc of the clear sample (filtered if necessary) with known F standards containing not more than 0.05 mg of F (waters containing sulfates, phosphates, Al salts, and other interfering substances vitiate the colorimetric comparison and give erroneous results).

According to the approximate F content, take a suitable quantity of water, make neutral to phenolphthalein indicator with 2.5% NaOH soln, and evaporate in porcelain to 5 cc. Transfer to the Claissen flask, using not more than 25 cc of  $H_2O$ .

## 25

## ISOLATION

Put into the Claissen flask 20-30 glass beads and place the flask on an asbestos mat with an opening large enough to expose about one-fourth of the flask to the flame. Close the straight neck of the flask with a two-holed rubber stopper thru which pass a thermometer and a small separatory funnel, the outlet of which is constricted to a diameter of about 2 mm. Have the thermometer and the funnel extend almost to the bottom of the flask. Close the other neck of the flask with a solid rubber stopper. Connect the flask with a water condenser, add 7 cc of  $H_2SO_4$  thru the separatory funnel, mix, and distil. Keep the temp. at  $135^\circ \pm 3^\circ$  during the distillation, regulating it by addition of  $H_2O$  from the separatory funnel. Collect 200 cc of distillate in a volumetric flask.

(a) *Color standards.*—To each of 6 Nessler tubes add 1.0 cc  $\text{HNO}_3$  (1+9) and 1.00 cc (accurately measured) of the  $\text{TiCl}_3$  soln, then 0.0, 0.5, 1.0, 1.5, 2.0, and 2.5 cc of the standard F soln. Add 30 cc of  $\text{H}_2\text{O}$  and 2 cc of the peroxide soln, make to mark, and mix by inverting the tubes at least 5 times. Test the pH of one of the series and replace. This gives a series of standards reading 0.00 to 0.05 mg of F. If the tubes are kept stoppered and protected from intense sunlight, they will keep constant for 24 hours.

If trouble is experienced in matching the yellow colors add, before making to volume, 2.0–2.5 cc (amount varying with the analyst's color preference) of the  $\text{Cu}(\text{NO}_3)_2$  soln (not sulfate, which introduces an interference). The resultant color varies from yellowish green (no F) thru green to bluish green.

(b) *Sample tubes.*—Mix distillate, and filter, if necessary, on a good grade quantitative filter. (Certain qualitative filters, particularly when old, have been found to impart appreciable amounts of yellow color to the filtrate.)

Prepare one or more sample tubes as directed under (a) substituting for the standard F soln a suitable aliquot of the distillate. For greatest accuracy use an aliquot that contains as near as possible (*but not exceeding*) 0.05 mg of F.

*Note 1.*—The quantities of  $\text{HNO}_3$  mentioned are approximate. Each analyst is expected to determine the accurate figures applicable to his own supply. The pH of the sample and the standards must be the same (within  $\pm 0.02$ ), and both should be pH 1.5 or slightly lower.

*Note 2.*—The limits of accuracy of the other reagents are:

	cc	cc
Titanium soln.....	1	$\pm 0.01$
Hydrogen peroxide.....	2	$\pm 0.02$
Copper nitrate.....	2	$\pm 0.02$

(c) *Color comparisons.*

(1) *Standard series method.*—When the proper amount of  $\text{HNO}_3$  (1+9) has been found giving the same pH in the sample tube as that in the color standards, make up a new sample tube and compare with the standards. Uniform, moderate illumination, a minimum of color interference by shadows or surrounding objects, and minimum of eye fatigue are necessary for uniform results. A three-compartment box comparator can easily be made and is a handy instrument.

(2) *Duplication method.*—Prepare a sample and one standard tube as described in (a), except to add no F to the standard tube, and to make it to a volume of approximately 48 cc. Titrate into the standard tube a F soln containing 0.02 mg of F per cc, until colors of sample and standard match, mixing *both* tubes by inverting 5 times after each addition. After taking the final buret reading, add 0.25 cc of F soln (0.005 mg F). A distinct color change should be visible.

Regardless of whether the standard series or the duplication method of comparison is used, the amount of F present in the aliquot taken *must not exceed* 0.05 mg. Above 0.05 mg of F the change in color per unit of F is less than below this limit. Hence, whenever a sample aliquot is found to contain more than 0.05 mg of F, it is *absolutely necessary* to repeat this determination on another aliquot small enough to bring the F content of the tube within the 0.05 mg limit.

## OXYGEN REQUIRED

*Method I.—Official*

27

## REAGENTS

(a) *Standard potassium permanganate soln.*—Dissolve 0.395 g of  $\text{KMnO}_4$  in 1 liter of  $\text{H}_2\text{O}$ . Each cc has 0.1 mg of O available for oxidation.

(b) *Standard oxalic acid soln.*—Dissolve 0.788 g of crystallized oxalic acid in 1 liter of  $\text{H}_2\text{O}$ .

Determine the value of the oxalic acid in terms of the permanganate by boiling 10 cc of the oxalic acid and 200 cc of redistilled  $\text{H}_2\text{O}$  (prepared by treating distilled  $\text{H}_2\text{O}$  with alkaline permanganate and distilling) with 10 cc of  $\text{H}_2\text{SO}_4$  (1+3) and titrating, while still boiling, with the standard permanganate to the appearance of a pink color.

28

## DETERMINATION

Add 10 cc of  $\text{H}_2\text{SO}_4$  (1+3) to 200 cc of the sample in a porcelain dish and heat to boiling. Add from a buret the standard permanganate until the  $\text{H}_2\text{O}$  is distinctly red and boil for 10 min., adding more of the standard permanganate from time to time to maintain the red color. Add 10 cc of the standard oxalic acid and titrate back with the standard permanganate to a pink color. From the total number of cc of permanganate used, subtract the number of cc equivalent to 10 cc of the oxalic acid. The result is the number of cc of the permanganate required for 200 cc of the  $\text{H}_2\text{O}$ . Correct for sulfides, nitrites, and ferrous salts, if present, by subtracting the number of cc of the standard permanganate absorbed by another 200 cc portion of the sample when treated as above, except to make the digestion at room temp. and for a period of 3 min.

*Method II.—Official*

(To be used when the chloride content of the sample is high.)

29

## REAGENTS

*Sodium hydroxide soln.*—Dissolve 50 g of  $\text{NaOH}$  in  $\text{H}_2\text{O}$ , cool, and make to 100 cc. The other reagents and solns used are described under 22.

30

## DETERMINATION

Introduce 100 cc of the  $\text{H}_2\text{O}$  to be examined into a 300 cc flask, add 0.5 cc of the  $\text{NaOH}$  soln and 10 cc of the permanganate, boil for 10 min., allow to cool to 50–60°, and add 5 cc of  $\text{H}_2\text{SO}_4$  (1+3) and 10 cc of the standard oxalic acid. As soon as the liquid has become perfectly colorless, and while constantly agitating, cautiously add the standard permanganate from a buret dropwise, until the liquid acquires a faint permanent redness. The permanganate used is the quantity required for decomposition of the organic matter in the 100 cc of the sample.

If 100 cc of the sample requires more than 4 cc of the permanganate for the oxidation of organic matter, make a second determination, using more of the permanganate and a correspondingly larger quantity of the  $\text{NaOH}$ , as undecomposed permanganate remaining after boiling must be at least twice as great as the quantity decomposed.

## DISSOLVED OXYGEN:

*Method I.—Official*

(When more than 0.1 mg of nitrite nitrogen per liter is present.)

31

## REAGENTS

(a) *Potassium permanganate soln.*—Dissolve 6.32 g of  $\text{KMnO}_4$  in  $\text{H}_2\text{O}$  and dilute to 1 liter.

(b) *Potassium oxalate soln.*—Dissolve 20 g of K oxalate in  $\text{H}_2\text{O}$  and dilute to 1 liter.

(c) *Manganous sulfate soln.*—Dissolve 480 g of  $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$  in  $\text{H}_2\text{O}$  and dilute to 1 liter.

(d) *Alkaline potassium iodide soln.*—Dissolve 500 g of  $\text{NaOH}$  and 150 g of  $\text{KI}$  in  $\text{H}_2\text{O}$  and dilute to 1 liter.

(e) *Sodium thiosulfate soln.*—0.025 *N*. Dissolve 6.205 g of chemically pure recrystallized  $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$  in  $\text{H}_2\text{O}$  and dilute to 1 liter with freshly boiled and cooled  $\text{H}_2\text{O}$ . 1 cc = 0.2 mg of O or 0.1400 cc of O at  $0^\circ$  and 760 mm pressure. Inasmuch as this soln is not permanent it should be standardized occasionally against a 0.025 *N* soln of  $\text{K}_2\text{Cr}_2\text{O}_7$ .

32

## COLLECTION OF SAMPLE

Collect the sample in a narrow-necked glass-stoppered bottle of 250–270 cc capacity by means of an apparatus designed to avoid the entrainment or absorption of any O from the atmosphere. Note the temp.

33

## DETERMINATION

Remove the stopper from the bottle and add 0.7 cc of  $\text{H}_2\text{SO}_4$  and then 1 cc of the  $\text{KMnO}_4$  soln. Introduce these and all other reagents by pipet under the surface of the liquid. Insert the stopper and mix by inverting the bottle several times. If a noticeable excess of  $\text{KMnO}_4$  is not present after 20 min., again add 1 cc of the permanganate soln; if this is still insufficient, use a stronger permanganate soln. After 20 min. destroy the excess of permanganate by adding 1 cc of the K oxalate soln, re-stopper the bottle at once, and mix its contents. Add 1 cc of the  $\text{MnSO}_4$  soln and 3 cc of the alkaline KI soln. Allow the precipitate to settle. Add 1 cc of  $\text{H}_2\text{SO}_4$  and mix by shaking. Transfer 200 cc of the contents of the bottle to a flask and titrate with the 0.025 *N*  $\text{Na}_2\text{S}_2\text{O}_3$ , using a few cc of starch indicator, VI, 3(e), toward the end of the titration. Do not add the starch soln until the color has become faint yellow. Titrate until the blue color disappears. Report the results in mg per liter; if desired, report results also as percentage of saturation.<sup>5</sup>

34

*Method II.—Official*

(When less than 0.1 mg of nitrite nitrogen per liter is present.)

For reagents and collection of sample, see 31 and 32. Remove the stopper from the bottle and proceed as directed under 33, beginning "Add 1 cc of the  $\text{MnSO}_4$  soln."

## LEAD\*

(When present in small quantities.)

*Method I.—Tentative*

(Coloring matter, iron, lead, copper, and zinc present.)

35

## REAGENTS

(a) *Ammonium acetate soln.*—Dissolve 200 g of  $\text{NH}_4$  acetate in  $\text{H}_2\text{O}$  and dilute to 500 cc. The soln should be practically colorless.

(b) *Dilute ammonium acetate soln.*—Dilute 50 cc of (a) to 500 cc.

(c) *Standard lead soln.*—Add  $\text{H}_2\text{SO}_4$  in slight excess to a 10% soln of Pb acetate. Filter off the  $\text{PbSO}_4$  and wash free from acid with  $\text{H}_2\text{O}$ . Dissolve the  $\text{PbSO}_4$  in the  $\text{NH}_4$  acetate soln, (a), dilute to a definite volume, and determine the Pb as  $\text{PbCrO}_4$  by precipitating with  $\text{K}_2\text{CrO}_4$  soln (cf. VI, 37). Dilute the stock soln so that 1 cc will contain 0.1 mg of Pb.

36

## REMOVAL OF COLOR

Acidify 0.5–2 liters of the sample with  $\text{HCl}$  (1+1) and concentrate in a porcelain casserole to a volume of about 75 cc by heating slowly over an open flame. Add sufficient  $\text{NH}_4\text{Cl}$  (about 2 g) to hold Mg in soln and assist in the separation of the sulfides. Add about 1 cc of  $\text{NH}_4\text{OH}$  in excess and saturate with  $\text{H}_2\text{S}$ . Cover the dish, allow to stand about 2 hours, add more of the  $\text{NH}_4\text{OH}$  and  $\text{H}_2\text{S}$ , boil a few minutes, let the precipitate settle, filter, and wash the precipitate once with hot  $\text{H}_2\text{O}$ . (The precipitate will contain all the Fe, Pb, Cu, and Zn, and the coloring matter will be in the filtrate.) Place the filter and precipitate in a small porcelain casserole, add 30 cc of  $\text{HNO}_3$  (1+3) and boil. Filter, wash free from acid, and cool the filtrate (soln A).

37

## DETERMINATION

Add to soln A, under 36, 5 cc of  $\text{H}_2\text{SO}_4$  (1+1), evaporate nearly to dryness, and heat cautiously until copious fumes of  $\text{SO}_3$  are given off. Cool, wash down the sides with a little  $\text{H}_2\text{O}$ , and repeat evaporation and heating. Transfer to a beaker with the aid of  $\text{H}_2\text{O}$ , add an equal volume of 95% alcohol, and let stand overnight. Filter off the  $\text{PbSO}_4$  and wash with dilute alcohol, 50% by volume, until free from Fe. Collect the filtrate, which contains Fe, Cu, and Zn, in a 250 cc beaker (soln B). Digest the filter containing the  $\text{PbSO}_4$  in a small porcelain casserole with about 40 cc of the warm  $\text{NH}_4$  acetate soln, filter, and wash once or twice with the warm dilute  $\text{NH}_4$  acetate soln and twice with  $\text{H}_2\text{O}$ . Dilute the filtrate to definite volume. To an aliquot portion add freshly prepared  $\text{H}_2\text{S}$  water and a few drops of acetic acid (1+1). Compare the color obtained with a set of standards made by treating various quantities of the standard Pb soln with  $\text{H}_2\text{S}$  water.

38

*Method II.—Tentative*

(Coloring matter present; iron present to extent of 1 mg or less in quantity of sample taken for analysis; copper and zinc absent.)

Remove coloring matter as directed under 36. Add to soln A 5 cc of  $\text{H}_2\text{SO}_4$  (1+1), evaporate nearly to dryness, and heat cautiously until copious fumes of  $\text{SO}_3$  are given off. Cool, wash down the sides with a little  $\text{H}_2\text{O}$ , and repeat evaporation and heating. Transfer to a beaker with the aid of  $\text{H}_2\text{O}$ , add 25–40 cc of the  $\text{NH}_4$  acetate

soln, 35, heat to boiling, and precipitate the Fe with  $\text{NH}_4\text{OH}$ . Filter and wash with the dilute  $\text{NH}_4$  acetate soln and  $\text{H}_2\text{O}$ . Acidify the filtrate slightly with acetic acid (1+1) and determine Pb colorimetrically in the filtrate by the addition of freshly prepared  $\text{H}_2\text{S}$  water as directed under 37.

39

*Method III.—Tentative*

(Coloring matter, iron, copper, and zinc absent.)

Add 5 cc of  $\text{H}_2\text{SO}_4$  (1+1) to 0.5–2 liters of the sample, evaporate nearly to dryness, and heat until copious fumes of  $\text{SO}_3$  are given off. Transfer to a beaker with the aid of  $\text{H}_2\text{O}$ , add 25–40 cc of the  $\text{NH}_4$  acetate soln, 35(a), and determine Pb colorimetrically by the addition of  $\text{H}_2\text{S}$  water as directed under 37.

40

*Method IV.—Tentative*

(Coloring matter absent: iron, lead, copper, and zinc present.)

Add 5 cc of  $\text{H}_2\text{SO}_4$  (1+1) to 0.5–2 liters of the sample, evaporate nearly to dryness, and heat until copious fumes of  $\text{SO}_3$  are given off. Filter off the  $\text{PbSO}_4$  and proceed as directed under 37.

## COPPER—TENTATIVE

(When present in small quantities.)

41

## REAGENTS

(a) *Ammonium nitrate soln.*—Dissolve 10 g of  $\text{NH}_4\text{NO}_3$  in  $\text{H}_2\text{O}$  and dilute to 100 cc.

(b) *Potassium ferrocyanide soln.*—Dissolve 3.5 g of  $\text{K}_4\text{Fe}(\text{CN})_6 \cdot 3\text{H}_2\text{O}$  in  $\text{H}_2\text{O}$  and dilute to 100 cc. This soln should be freshly prepared.

(c) *Standard copper soln.*—Dissolve about 20 g of  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  in  $\text{H}_2\text{O}$ , add 1 cc of  $\text{H}_2\text{SO}_4$ , and dilute to 500 cc. Determine the Cu in 50 cc of this soln as  $\text{CuO}$  by precipitation with  $\text{KOH}$  soln. Dilute the stock soln so that 1 cc contains 0.1 mg of Cu.

42

## DETERMINATION

Boil the moderately acid filtrate (soln B), which contains Fe, Cu, and Zn, to remove alcohol; adjust the soln to a volume of about 200 cc; and add 1 g of  $\text{NH}_4\text{Cl}$ . Heat to boiling, saturate with  $\text{H}_2\text{S}$  gas, and boil to remove precipitated S. Cover beaker, let stand about 2 hours or until supernatant liquid becomes clear, filter, and wash the  $\text{CuS}$  without intermission with  $\text{H}_2\text{O}$  containing  $\text{H}_2\text{S}$ . Collect the filtrate in a porcelain casserole (soln C). Dissolve the precipitate of copper sulfide in hot  $\text{HNO}_3$  (1+3). Cool, add a few drops of phenolphthalein indicator, and make the soln slightly alkaline with  $\text{NH}_4\text{OH}$  added carefully from a dropping bottle. Add 10 cc of the  $\text{NH}_4\text{NO}_3$  soln, adjust the volume to 100 cc, and boil gently until a test with red litmus paper shows the soln to be neutral. Filter the soln to remove any Fe that may be present and adjust the filtrate to a volume of 100 cc. To an aliquot add 3 drops of the  $\text{K}_4\text{Fe}(\text{CN})_6$  soln. Compare the color obtained with standards containing 0.1, 0.2, 0.3, 0.4, and 0.5 mg of Cu. Prepare these standards by measuring corresponding quantities of the standard Cu soln; adding phenolphthalein indicator, a slight excess of  $\text{NH}_4\text{OH}$ , and 10 cc of the  $\text{NH}_4\text{NO}_3$ ; boiling the soln until neutral to red litmus, cooling, and adding 3 drops of the  $\text{K}_4\text{Fe}(\text{CN})_6$  soln. Make the colorimetric comparison in 100 cc Nessler jars.

## ZINC—TENTATIVE

(When present in small quantities.)

43

## REAGENTS

(a) *Citric acid soln.*—Dissolve 50 g of citric acid crystals ( $C_6H_8O_7 \cdot H_2O$ ) in  $H_2O$  and dilute to 100 cc.

(b) *Ammonium thiocyanate soln.*—Dissolve 20 g of  $(NH_4)SCN$  in  $H_2O$  and dilute to 1 liter.

(c) *Standard zinc soln.*—Dissolve pure Zn in HCl and dilute so that 1 cc contains 0.1 mg of Zn.

(d) *Potassium ferrocyanide soln.*—Dissolve 3.5 g of  $K_4Fe(CN)_6 \cdot 3H_2O$  in  $H_2O$  and dilute to 100 cc.

44

## DETERMINATION

Boil the acid filtrate (soln C) from the copper sulfide precipitation to remove  $H_2S$ , cool, neutralize with  $NH_4OH$ , and add 10 cc of the citric acid soln. Heat to boiling and if no Ca citrate separates add small quantities of powdered  $CaCO_3$  until a precipitate of about 1 g of Ca citrate is formed. Pass  $H_2S$  thru the soln until it is cool. Let stand several hours, part of the time on a steam bath, until the supernatant liquid is clear.

Filter, wash with the  $(NH_4)SCN$  soln and dissolve the precipitate on the filter with hot HCl (1+9). If the filtrate is reddish in color, reprecipitate the Zn as before. Dispel turbidity of the filtrate due to colloidal S by boiling. When the filtrate is clear and colorless, dilute an aliquot to 45 cc in a 50 cc Nessler jar. Add 5 cc of the  $K_4Fe(CN)_6$  soln, mix quickly, and compare the turbidity with standard Zn solns by viewing longitudinally the jars held over a sheet of fine print. Prepare the standards by mixing definite volumes of the standard Zn soln, 3 cc of HCl,  $H_2O$  to make 45 cc, and 5 cc of the  $K_4Fe(CN)_6$  soln. The unknown soln should contain a volume of acid equivalent to that in the standards. Do not use Zn borosilicate glassware in this determination.

## MINERAL WATER

45

## SPECIFIC GRAVITY—OFFICIAL

Determine specific gravity at  $20/20^\circ$  by means of a pycnometer (XIV, 3).

46

## SOLIDS IN SOLUTION OFFICIAL

Proceed as directed under 7.

47

## IGNITED RESIDUE—OFFICIAL

Proceed as directed under 9.

48

## NITROGEN IN THE FORM OF FREE AND ALBUMINOID AMMONIA—OFFICIAL

Proceed as directed under 11 or 13.

49

## NITROGEN IN THE FORM OF NITRITE—OFFICIAL

Proceed as directed under 15.

50

## NITROGEN IN THE FORM OF NITRATE—OFFICIAL

Proceed as directed under 17 or 19.

51

## CHLORIDE—OFFICIAL

Proceed as directed under 21.

## HYDROGEN SULFIDE—OFFICIAL

52

## REAGENTS

(a) *Iodine soln.*—0.02 *N*. Dissolve 10 g of KI (free from iodic acid) in a liter flask, using as little  $H_2O$  as possible. Add 2.54 g of resublimed I and dissolve by shaking. Dilute to the mark with  $H_2O$ . Standardize against a  $Na_2S_2O_3$  soln that has been recently standardized against a  $K_2Cr_2O_7$  soln.

(b) *Iodine soln.*—0.01 *N*. Mix equal volumes of (a) and boiled  $H_2O$ . Standardize against a  $Na_2S_2O_3$  soln as directed under (a).

53

## DETERMINATION

Transfer a quantity of the sample to a graduated vessel by means of a siphon and add a few drops of phenolphthalein indicator. If alkaline, add HCl until the pink color of the indicator disappears. Add starch indicator, and with careful stirring titrate with I soln, (a) or (b), until a permanent blue color appears. Correct for the quantity of I soln needed to give an equally blue color. From the corrected quantity of I soln used, calculate the approximate quantity of  $H_2S$  present. For accurate determinations siphon 100–500 cc of the sample, according to the quantity of  $H_2S$  present, into a graduated vessel, keeping the outlet of the siphon below the liquid. Add immediately a sufficient quantity of HCl, calculated from the approximate determination, to make neutral to phenolphthalein indicator. Mix carefully with a bent glass rod and without delay add about 0.5 cc less I reagent (a) or (b) than is needed to combine with the  $H_2S$  present.

Add 5 cc of starch indicator, VI, 3(e), and finish the titration with I soln dropwise with stirring until a blue color remains permanently. Correct for the quantity of I soln needed to give an equally blue color when the same quantity of starch soln is added to an approximately equal volume of boiled  $H_2O$ . If possible, make several determinations and take an average. Standardize reagents (a) and (b) frequently.

54

## FREE CARBON DIOXIDE—TENTATIVE

If the sample reacts acid to phenolphthalein and alkaline to methyl orange, titrate 100 cc with 0.05 *N*  $Na_2CO_3$  (free from bicarbonate) until the soln is neutral to phenolphthalein. The number of cc used multiplied by 1.1 gives the mg of free  $CO_2$  in 100 cc. Express the results in mg per liter.

55

## CARBONIC AND BICARBONIC ACIDS—OFFICIAL

To 100 cc of the sample add a few drops of phenolphthalein, and if a pink color is produced titrate with 0.05 *N* HCl or  $H_2SO_4$ , adding a drop every 2–3 seconds until the pink color disappears. Multiply the buret reading by the factor 3 to obtain the mg of  $CO_3$  ion in 100 cc. To the colorless soln from this titration, or to the original soln if no color is produced with phenolphthalein, add 1 or 2 drops of methyl orange, continue the titration without refilling the buret, and note the total reading. If  $CO_2$  is absent, multiply the total buret reading by the factor 3.05 to obtain the value of  $HCO_3$  ion in mg per 100 cc. If  $CO_2$  is present, multiply the reading with phenolphthalein by 2 and subtract from the total reading of the buret. Multiply the difference by 3.05 to obtain the  $HCO_3$  ion in mg per 100 cc. Express the results as mg per liter.



56

## SILICA—OFFICIAL

Make a preliminary examination, using 100–250 cc of the sample, to determine the approximate quantity of Ca and Mg present, in order to ascertain the quantity of sample to be evaporated for the final analysis.

Evaporate the quantity sufficient to yield 0.1–0.6 g of CaO or 0.1–1 g of  $Mg_2P_2O_7$  (usually 1–5 liters). Acidify the  $H_2O$  with HCl and evaporate on a steam bath to dryness in a Pt dish. Continue the drying for about an hour. Thoroughly moisten the residue with 5–10 cc of HCl. Allow to stand 10–15 min. and add sufficient  $H_2O$  to bring the soluble salts into soln. Heat on a steam bath until soln of the salts is effected. Filter to remove most of the  $SiO_2$  and wash thoroughly with hot  $H_2O$ . Evaporate the filtrate to dryness and treat the residue with 5 cc of HCl and sufficient  $H_2O$  to effect soln of the soluble salts, as before. Heat, filter, and wash thoroughly with hot  $H_2O$ . Designate the filtrate as A. Transfer the 2 residues to a Pt crucible, ignite, heat over a blast lamp, and weigh. Moistened the contents of the crucible with a few drops of  $H_2O$ . Add a few drops of  $H_2SO_4$  and a few cc of HF and evaporate on a steam bath under a hood. Repeat the treatment if all the  $SiO_2$  is not volatilized. Dry carefully on a hot plate, ignite, heat over a blast lamp, and weigh. The difference between the 2 weights is the weight of the  $SiO_2$ . Add the weight of the residue to that of the total  $Al_2O_3$  and  $Fe_2O_3$  obtained under 57. (If the residue weighs more than 0.5 mg,  $BaSO_4$  may be found in it when Ba is present in the  $H_2O$ . If so, make the necessary correction and add to the weight of the total  $Fe_2O_3$  and  $Al_2O_3$  under 57.)

57

## IRON AND ALUMINUM—OFFICIAL

Concentrate A, under 56, to about 200 cc; while still hot, add  $NH_4OH$  slowly, with constant stirring, until alkaline to methyl orange. Boil, filter, and wash 2 or 3 times with hot  $H_2O$ . Dissolve the precipitate in hot HCl (1+1). Dilute to approximately 25 cc, boil, and again precipitate with  $NH_4OH$ . Filter, wash thoroughly with hot  $H_2O$ , dry, ignite, and weigh as  $Al_2O_3$  and  $Fe_2O_3$ . (In the presence of  $H_3PO_4$ , the weight of this residue must be corrected for the  $P_2O_5$  equivalent to the  $H_3PO_4$  found under 70, allowance being made for the difference in the volumes of the  $H_2O$  used for these determinations.) Designate the filtrate as B.

## IRON

58

*Colorimetric Method—Official*

(If the quantity of iron is less than 1 mg. Not applicable in the presence of phosphates.)

Fuse in a Pt crucible the ignited precipitate of  $Fe_2O_3$  and  $Al_2O_3$  with fused  $KHSO_4$ , dissolve in  $H_2O$ , and precipitate the Fe and Al with  $NH_4OH$ . Dissolve the precipitate on the filter paper in HCl and  $HNO_3$ , dilute the soln, add 5%  $(NH_4)SCN$  soln, and compare the color developed with that of calibrated color disks or standards containing known quantities of Fe.

59

*Volumetric Method—Official*

Fuse in a Pt crucible the residue of  $Fe_2O_3$  and  $Al_2O_3$  with fused  $KHSO_4$ . This fusion takes but a few minutes and must not be continued beyond the time actually needed. When the fusion is completed, set the crucible aside and allow to cool. Add  $H_2SO_4$  (1+4) and heat the crucible until the fused mass is dissolved. Evaporate on a steam bath as far as possible; then heat gradually until copious fumes of  $SO_2$  are given off. Dissolve in  $H_2O$  and allow to stand on the steam bath. Cool, transfer to

an Erlenmeyer flask, and make up to such a volume that the soln does not contain more than 2.5% of free  $\text{H}_2\text{SO}_4$ . Pass  $\text{H}_2\text{S}$  thru the soln to reduce the Fe and precipitate any Pt contaminating the residue from the fusion. (Zn may be used instead of  $\text{H}_2\text{S}$  for reducing the Fe.) Filter, wash, and again pass  $\text{H}_2\text{S}$  thru the soln so that all the Fe will be reduced. Expel the  $\text{H}_2\text{S}$  by boiling, at the same time passing a current of  $\text{CO}_2$  thru the soln. Test the escaping gas with Pb acetate paper to ascertain the complete removal of  $\text{H}_2\text{S}$ . Discontinue boiling and let the flask cool without discontinuing the current of  $\text{CO}_2$ . Titrate the reduced Fe with a standard permanganate soln, 1 cc = 1 mg of Fe, and calculate as Fe.

60

## ALUMINUM—OFFICIAL

To obtain the weight of  $\text{Al}_2\text{O}_3$ , in the absence of phosphates, subtract from the weight of  $\text{Fe}_2\text{O}_3$  and  $\text{Al}_2\text{O}_3$ , 57, the Fe, 58 or 59, calculated to  $\text{Fe}_2\text{O}_3$ . Calculate to Al.

61

## CALCIUM—OFFICIAL

Concentrate B, under 57, to 150–200 cc, and to this soln, containing an equivalent of not more than 0.6 g of CaO, or 1 g of  $\text{Mg}_2\text{P}_2\text{O}_7$ , add 1–2 g of oxalic acid and sufficient HCl (1+1) to clear the soln. Heat to boiling and neutralize with  $\text{NH}_4\text{OH}$ , stirring constantly. Add the  $\text{NH}_4\text{OH}$  in slight excess and allow to stand 3 hours in a warm place. Filter off the supernatant liquid and wash the precipitate once or twice by decantation with 1%  $\text{NH}_4$  oxalate soln. Dissolve the precipitate in HCl (1+1), dilute to 100–200 cc, add a little more oxalic acid, and precipitate as above. After allowing to stand 3 hours, filter, wash with the 1%  $\text{NH}_4$  oxalate soln, dry, ignite, heat over a blast lamp, and weigh as CaO and SrO. Subtract from this weight, the weight of SrO equivalent to the Sr under 62. The difference is the weight of CaO. Calculate to Ca. Designate the combined filtrates and washings as C.

As a check on the CaO, evaporate to dryness the filtrate from the  $\text{Sr}(\text{NO}_3)_2$  under 62, beginning with "Filter, and wash with ether-alcohol mixture, etc."; dissolve the  $\text{Ca}(\text{NO}_3)_2$  in  $\text{H}_2\text{O}$ , precipitate as oxalate, filter, wash, ignite, and weigh as CaO.

62

STRONTIUM<sup>11</sup>—TENTATIVE

Dissolve the oxides under 61 in  $\text{HNO}_3$  (1+1) and test with the spectroscope for Sr. If Sr is present, transfer the  $\text{HNO}_3$  soln to a small Erlenmeyer flask. Evaporate nearly to dryness over a low flame and heat in an air bath at 150–160° for 1–2 hours after the  $\text{H}_2\text{O}$  is evaporated. Break up the dried material with a stirring rod and add 10–15 cc of a mixture of equal parts of absolute alcohol and ether to dissolve the  $\text{Ca}(\text{NO}_3)_2$ . Cork the flask and allow to stand with frequent shaking for 2 hours or longer. Decant the soln thru a 5.5 cm filter, reserving the filtrate. Wash the residue several times by decantation with small portions of ether-alcohol soln. Dry the residue and the filter paper and wash the filter paper repeatedly with small portions of hot  $\text{H}_2\text{O}$ , collecting the filtrate in the flask containing the main portion of the  $\text{Sr}(\text{NO}_3)_2$  residue. Add 1 or 2 drops of  $\text{HNO}_3$  (1+1), evaporate, dry, pulverize, and treat with 10–15 cc of the ether-alcohol mixture. Cork the flask and let stand about 12 hours with occasional shaking. Filter, and wash with ether-alcohol mixture until a few drops of the filtrate evaporated on a watch-glass leave practically no residue. Dry the paper and precipitate. Dissolve the  $\text{Sr}(\text{NO}_3)_2$  in a few cc of hot  $\text{H}_2\text{O}$ . Add a few drops of  $\text{H}_2\text{SO}_4$  and then a volume of alcohol equal to the volume of the soln and allow to stand 12 hours. Filter, ignite, weigh as  $\text{SrSO}_4$ , and calculate to Sr. Test spectroscopically for Ca and Ba. If these elements are present, determine the quantity and make the necessary correction.

## 63

## MAGNESIUM—OFFICIAL

Concentrate *C*, under 61, to about 200 cc and add 2–3 g of  $(\text{NH}_4)_2\text{HPO}_4$  and sufficient  $\text{HCl}$  (1+1) to clear the soln when all the  $(\text{NH}_4)_2\text{HPO}_4$  is dissolved. When cold, make slightly alkaline with  $\text{NH}_4\text{OH}$ , stirring constantly. Add 1–2 cc excess of the  $\text{NH}_4\text{OH}$  and allow to stand about 12 hours. Filter off the supernatant liquid and wash 3 or 4 times by decantation with a soln of  $\text{NH}_4\text{OH}$  (1+10). Dissolve the precipitate in  $\text{HCl}$  (1+1), dilute to about 150 cc, add a little  $(\text{NH}_4)_2\text{HPO}_4$ , and precipitate with  $\text{NH}_4\text{OH}$  as before. Allow to stand 6–12 hours, filter, wash free from chlorides with  $\text{NH}_4\text{OH}$  (1+10), ignite, heat over a blast lamp, and weigh as  $\text{Mg}_2\text{P}_2\text{O}_7$ . Calculate to  $\text{Mg}$ .

## 64

## SULFURIC ACID—OFFICIAL

Make a preliminary examination, using 100–250 cc of the sample, to determine the approximate quantity of sulfates. (The alkali salts present can be approximated by calculating the quantity of  $\text{Na}$  necessary to combine with the excess of acids— $\text{HCl}$ ,  $\text{H}_2\text{SO}_4$ , and  $\text{H}_2\text{CO}_3$ —over the  $\text{Ca}$  and  $\text{Mg}$ .)

Take a quantity (usually 1–5 liters) sufficient to yield not more than 1 g of  $\text{BaSO}_4$ , and not more than 0.5 g of mixed chlorides. Acidify with  $\text{HCl}$  (1+1), evaporate to dryness in a Pt dish, and remove  $\text{SiO}_2$  by 2 evaporations as directed under 56, using not more than 2 cc of  $\text{HCl}$  for the final soln. Combine the filtrate and washings from the  $\text{SiO}_2$  determinations and concentrate to about 150–200 cc. Heat to boiling and precipitate with a slight excess of 10%  $\text{BaCl}_2$  soln, added very slowly and with constant stirring. Cover, and allow to stand on a steam bath about 12 hours. Filter, thoroly wash the precipitate of  $\text{BaSO}_4$  with hot  $\text{H}_2\text{O}$ , dry, ignite over a Bunsen burner, and weigh.

If the content of sulfate in the sample is unusually large, proceed as far as the concentration of the  $\text{SiO}_2$  filtrates, as directed above. Add 50 cc of  $\text{HCl}$ , heat to boiling, and precipitate with  $\text{BaCl}_2$  soln as before. Evaporate to dryness, wash the precipitate repeatedly by decantation, and filter. Complete the washing of the precipitate, ignite, and weigh. Calculate to the  $\text{SO}_4$  ion. Designate the filtrate as *E*.

## SODIUM, POTASSIUM, AND LITHIUM

*Ether Alcohol Method*<sup>12</sup>—Official

## 65

## PREPARATION OF THE MIXED CHLORIDES

Evaporate to dryness *E*, under 64, in a Pt dish and ignite the residue to faint redness to remove all traces of  $\text{NH}_4$  salts. Dissolve the residue in the dish in about 200 cc of  $\text{H}_2\text{O}$  and precipitate with milk of lime or a saturated soln of  $\text{Ba}(\text{OH})_2$ . Boil, allow to stand for 30 min., and filter off the insoluble  $\text{Mg}(\text{OH})_2$  and undissolved lime. Thoroly wash the precipitate with hot  $\text{H}_2\text{O}$  and combine the filtrate and washings. If the precipitate of  $\text{Mg}$  is large, dissolve it in a small quantity of  $\text{HCl}$ , evaporate to dryness, take up with  $\text{H}_2\text{O}$ , and precipitate as before. Concentrate the 2 filtrates and washings to 200–250 cc. Add  $\text{NH}_4\text{OH}$  and sufficient solid  $\text{NH}_4$  carbonate to precipitate the  $\text{Ca}$  and  $\text{Ba}$ . Allow to stand on a steam bath for 1–2 hours. Filter off the supernatant liquid, dissolve the precipitate in  $\text{HCl}$ , reprecipitate as above, and wash thoroly with hot  $\text{H}_2\text{O}$ . Evaporate the combined filtrates and washings to dryness and drive off the  $\text{NH}_4$  salts by gentle heat. Treat the residue with  $\text{H}_2\text{O}$ , pass thru a small filter, using as little wash  $\text{H}_2\text{O}$  as possible, evaporate to a small volume, and again precipitate with 1 or 2 drops of  $\text{NH}_4\text{OH}$  and 2 or 3 drops of saturated solns of  $\text{NH}_4$  carbonate and  $\text{NH}_4$  oxalate. If any precipitate appears,

filter and repeat the process. Evaporate the filtrate to dryness and drive off all  $\text{NH}_4$  salts by heating to faint redness in a Pt dish. Treat the residue with a little  $\text{H}_2\text{O}$ , filter into a small Pt dish, add a few drops of  $\text{HCl}$  (1+1), and evaporate to dryness. Dry in an oven, heat to faint redness, cool in a desiccator, and weigh the combined chlorides of K, Na, and Li. Repeat the heating to constant weight ( $x$ ). Dissolve the mixed chlorides in hot  $\text{H}_2\text{O}$ , filter, and wash. Return the filter paper and residue to the dish, dry, ignite, and weigh ( $y$ ). The difference between ( $x$ ) and ( $y$ ) is the weight of the mixed chlorides.

## 66

## DETERMINATION

Dissolve the mixed chlorides, obtained as directed under 65, in a minimum quantity of cold  $\text{H}_2\text{O}$  (about 1.5 cc will be more than sufficient for 0.5 g of the salts), introducing the soln into a tall 200 cc beaker. Add 1 drop of  $\text{HCl}$ , and then add *gradually* 20 cc of absolute alcohol, dropping the alcohol into the center of the beaker (not on the sides) while rotating the soln. (The  $\text{NaCl}$  and  $\text{KCl}$  should be precipitated in a perfectly uniform granular condition.) In a similar manner, add 60 cc of ether (sp. gr. 0.716–0.717 at  $25^\circ$ ) and allow the mixture to stand about 5 min. or until the precipitate is well agglomerated and the supernatant liquid almost clear, rotating the mixture occasionally during this period. Filter thru a weighed Gooch crucible into an Erlenmeyer flask by means of suction, using a bell jar arrangement, washing the beaker thoroly with a mixture of 1 part alcohol and 5 parts ether, and collecting all the precipitate on the Gooch with the aid of a rubber-tipped rod. After thoroly washing the precipitate on the Gooch, set the latter aside and rinse the funnel with alcohol-ether mixture to wash any adhering Li soln into the flask containing the filtrate. Evaporate the filtrate to dryness on a steam bath, using an air blast. Treat the residue with 10 cc of *absolute* alcohol, warming if necessary, so that practically all the residue dissolves. If a slight film remains on the bottom and sides of the flask, remove it with a rubber-tipped rod. Then, while rotating the soln in the flask, add 50 cc of ether (sp. gr. 0.716–0.717 at  $25^\circ$ ), followed by 1 drop of  $\text{HCl}$ . Allow to stand for 30 min., rotating the soln at frequent intervals. When the fine precipitate has agglomerated (only a very small quantity is usually precipitated), filter into a tall beaker by means of suction thru the Gooch crucible containing the first precipitate. Wash the combined precipitates with the ether-alcohol mixture, taking the same precautions as in the first precipitation. Dry the Gooch and its contents in an oven, ignite gently, cool, and weigh to obtain the combined weight of  $\text{NaCl}$  and  $\text{KCl}$ . Reserve crucible and contents for the determination of K.

Evaporate on a steam bath the ether-alcohol filtrate and washings containing the Li. Dissolve the residue in a little  $\text{H}_2\text{O}$ , add a slight excess of  $\text{H}_2\text{SO}_4$  (1+1), and transfer to a weighed porcelain or Pt dish. Evaporate as far as possible on a steam bath and then gently ignite the residue over a flame. (By placing the dish on a triangle over an asbestos gauze and using a low flame, the soln can be evaporated without spattering.) Finally ignite carefully over a full flame, cool, and weigh. If charring has occurred, repeat the ignition with  $\text{H}_2\text{SO}_4$ . Calculate to Li, using the factor 0.1263.

Remove the  $\text{KCl}$  and  $\text{NaCl}$  from the Gooch crucible by washing with 25–50 cc of hot  $\text{H}_2\text{O}$ , using suction, and collecting the filtrate in a porcelain dish. Add sufficient Pt soln, II, 42(b), to convert the  $\text{KCl}$  and  $\text{NaCl}$  to  $\text{K}_2\text{PtCl}_6$  and  $\text{Na}_2\text{PtCl}_6$  and evaporate to dryness. Treat the residue with 80% alcohol by volume, filter, and wash until the excess of  $\text{PtCl}_4$  and  $\text{Na}_2\text{PtCl}_6$  has been removed. Dry the filter and precipitate, dissolve the residue in hot  $\text{H}_2\text{O}$ , and transfer to a weighed Pt dish. Evaporate on a

steam bath, dry for 30 min. in an oven at 100°, cool, and weigh as  $K_2PtCl_6$ . Calculate to KCl, using the factor 0.3067, and to K, using the factor 0.1609.

Determine the weight of NaCl by subtracting the weight of KCl from the weight of combined KCl and NaCl. Calculate to Na, using the factor 0.3934.

#### BARIUM

It is not necessary to look for barium if sulfate is present in an appreciable quantity unless the  $H_2O$  contains a large quantity of bicarbonate or chloride, which may hold in soln a small quantity of both sulfate and barium.

#### Gravimetric Method<sup>13</sup>—Official

67

#### REAGENTS

(a) *Ammonium dichromate soln.*—Dissolve 100 g of the salt free from  $SO_4$  in  $H_2O$  and dilute to 1 liter.

(b) *Ammonium acetate soln.*—Dissolve 300 g of the salt in  $H_2O$ , neutralize with  $NH_4OH$ , and dilute to 1 liter.

(c) *Dilute ammonium acetate soln.*—Dilute 20 cc of (b) to 1 liter.

Reaction of acetate solns should be alkaline rather than acid.

68

#### DETERMINATION

Acidify a 1-5 liter portion of the sample with HCl and concentrate to about 200 cc. (If a precipitate forms, filter it off and examine for Ba.) Add about 0.5 g of  $NH_4Cl$  and precipitate the Fe and Al with  $NH_4OH$ . Boil, filter, and wash. To the filtrate add an excess of the  $NH_4$  acetate soln, 10 cc, keeping the total volume about 200 cc. Heat to boiling and add, with stirring, about 5 cc of the  $NH_4$  dichromate soln. Allow to settle and cool. Decant the clear liquid thru a filter and wash the precipitate by decantation with the dilute  $NH_4$  acetate soln until the filtrate is no longer perceptibly colored (100 cc of wash soln). Place the beaker under the funnel, dissolve the precipitate on the paper with warm  $HNO_3$  (1 + 1), using as little as possible, and wash the paper. Add a little more acid to dissolve the precipitate in the beaker, then  $NH_4OH$  until the precipitate which forms no longer redissolves. Heat to boiling; add with stirring, 10 cc of the  $NH_4$  acetate soln and 2 cc of the  $NH_4$  dichromate soln; allow to cool slowly and wash the precipitate by decantation with the dilute  $NH_4$  acetate soln. Dry the  $BaCrO_4$ , burn the filter separately, ignite moderately to constant weight, and weigh as  $BaCrO_4$ . Record as Ba, using the factor 0.54217.

69

#### Volumetric Method—Official

Proceed as directed under 68 thru "wash the precipitate by decantation with the dilute  $NH_4$  acetate soln" (after the second precipitation). Then proceed as follows:

Dissolve the precipitate in about 10 cc of HCl (1 + 1) and hot  $H_2O$ . Wash the filter, dilute the soln to about 400 cc, and add about 50 cc of a freshly prepared 10% soln of KI. Mix carefully and titrate the liberated I after 3 or 4 min. with 0.1 N  $Na_2S_2O_3$  soln. 1 cc of 0.1 N  $Na_2S_2O_3$  = 4.579 mg of Ba.

70

#### PHOSPHORIC ACID—OFFICIAL

Treat 500 cc of the sample, or a larger quantity if necessary, with about 10 cc of  $HNO_3$ , and evaporate in a porcelain dish nearly to dryness to drive off HCl. Treat the residue with  $H_2O$  and filter if necessary. Add  $NH_4OH$  to alkalinity and then just enough  $HNO_3$  to restore acidity. Add some solid  $NH_4NO_3$  and heat in a water

bath at a temp. of 45–50°. Add molybdate soln, II, 10(a), and keep at the above temp. for 30 min. If more than a trace of the yellow precipitate is present, filter and wash with recently boiled and cooled H<sub>2</sub>O until entirely free from nitric and molybdic acids. Transfer the precipitate and filter to a beaker, add a little H<sub>2</sub>O, and beat the paper and contents to a pulp. Dissolve the yellow precipitate in a small quantity of standard KOH, II, 10(b), add phenolphthalein indicator, II, 10(d), and titrate with the standard acid. From the data so obtained calculate the PO<sub>4</sub> ion to mg per liter.

#### 71 PREPARATION OF SAMPLE—MANGANESE, IODINE, BROMINE, ARSENIC, AND BORIC ACID

Evaporate 0.5–2 liters of the sample to dryness after the addition of small quantities of solid Na<sub>2</sub>CO<sub>3</sub>. Boil the residue thus obtained with H<sub>2</sub>O, transfer to a filter, and wash thoroly with hot H<sub>2</sub>O. Use the residue remaining on the filter for the determination of Mn. Dilute the alkaline filtrate to a definite volume and use for the determination of I, Br, As, and H<sub>3</sub>BO<sub>3</sub>.

#### MANGANESE

##### I. *Persulfate Method—Official*

72

#### REAGENTS

- (a) *Silver nitrate soln.*—Dissolve 2 g of AgNO<sub>3</sub> in H<sub>2</sub>O and dilute to 1 liter.  
 (b) *Standard manganous sulfate soln.*—Dissolve 0.2877 g of pure KMnO<sub>4</sub> in about 100 cc of H<sub>2</sub>O, acidify the soln with H<sub>2</sub>SO<sub>4</sub> (1+1), and slowly heat to boiling. Add slowly a sufficient quantity of a 10% oxalic acid soln to discharge the color. Cool, and dilute to 1 liter. 1 cc of this soln = 0.1 mg of Mn.

73

#### DETERMINATION

Dissolve the insoluble residue, 71, in an excess of HNO<sub>3</sub> (1+1), evaporate to dryness, treat with H<sub>2</sub>O, and add about 1 cc of HNO<sub>3</sub> and a little of the AgNO<sub>3</sub> soln. If a precipitate of AgCl appears, add more of the AgNO<sub>3</sub> until all the Cl is precipitated. Add an excess of about 10 cc of the AgNO<sub>3</sub> soln for each mg of Mn present in the sample. Filter, add 1 g of NH<sub>4</sub> persulfate to the filtrate, and place the beaker or flask containing the soln on a steam bath until a pink color develops (usually about 20 min.). Compare the color developed with standards similarly prepared by treating solns containing known quantities of the standard MnSO<sub>4</sub> with the dilute HNO<sub>3</sub>, AgNO<sub>3</sub> soln, and NH<sub>4</sub> persulfate.

##### II. *Bismuthate Method<sup>14</sup>—Official*

74

#### REAGENTS

- (a) *Nitric acid.*—(1+4). Free from brown oxide of N by aeration.  
 (b) *Dilute sulfuric acid.*—Dilute 25 cc of H<sub>2</sub>SO<sub>4</sub> to 1 liter with H<sub>2</sub>O. Add enough KMnO<sub>4</sub> soln to color the dilute acid faintly.  
 (c) *Standard manganous sulfate soln.*—Prepare as directed under 72(b). A soln of KMnO<sub>4</sub> may be used in place of the MnSO<sub>4</sub> soln. To prepare it dissolve 0.2877 g of KMnO<sub>4</sub> in H<sub>2</sub>O and dilute to 1 liter.

75

#### DETERMINATION

Remove Cl by 2 or more evaporations with H<sub>2</sub>SO<sub>4</sub> (1+1) from a quantity of the sample that contains 1 mg or less of Mn. The residue obtained under 71 may be used

in place of a fresh sample by dissolving it in an excess of  $\text{HNO}_3$  (1+4), adding the dilute  $\text{H}_2\text{SO}_4$ , and removing Cl by 2 or more evaporations. In either case, volatilize the  $\text{H}_2\text{SO}_4$  and ignite the residue at a low heat (less than  $500^\circ$ ). Dissolve in 40 cc of  $\text{HNO}_3$  (1+3), add about 0.5 g of the Na bismuthate, and heat until the permanganate color disappears. Add a few drops of a soln of  $\text{NH}_4$  or Na bisulfite to clear the soln and again boil to expel oxides of N. Remove from the source of heat, cool to  $20^\circ$ , again add 0.5 g of the Na bismuthate, and stir. When the maximum permanganate color has developed, filter thru an alundum or Gooch crucible containing an asbestos mat that has been ignited, treated with a 4% soln of  $\text{KMnO}_4$ , and washed with  $\text{H}_2\text{O}$ . Wash the precipitate with  $\text{H}_2\text{SO}_4$  (1+9) until the washings are colorless. Transfer the filtrate to a colorimeter tube. Compare the color developed with standards similarly prepared by treating solns containing known quantities of the standard  $\text{MnSO}_4$  with the dilute  $\text{HNO}_3$ ,  $\text{NH}_4$  or Na bisulfite soln, and Na bismuthate. The color may also be compared with that of standards prepared from the  $\text{KMnO}_4$  soln by diluting portions of 0.2, 0.4, 0.6 cc, etc., of the permanganate soln with the dilute  $\text{H}_2\text{SO}_4$  to the same volume as the filtrate.

#### IODIDE AND BROMIDE—TENTATIVE

(This method is qualitative and approximately quantitative. For accurate quantitative methods for iodides and bromides, see 102, 105, 109, and 112.)

#### 76

##### REAGENTS

(a) *Sodium hydroxide soln.*—Dissolve 10 g of NaOH in  $\text{H}_2\text{O}$ , cool, and dilute to 100 cc.

(b) *Sodium nitrite soln.*—Dissolve 2 g of  $\text{NaNO}_2$  in  $\text{H}_2\text{O}$  and dilute to 1 liter.

#### 77

##### DETERMINATION

Evaporate to dryness an aliquot of the alkaline filtrate, 71; add 2–3 cc of  $\text{H}_2\text{O}$  to dissolve the residue and enough 95% alcohol to make the percentage of alcohol about 90. (This precipitates the chlorides.) Heat to boiling, filter, and repeat the preceding soln and precipitation once or twice. Add 2 or 3 drops of the NaOH soln to the combined alcoholic filtrates and evaporate to dryness. Dissolve this last residue in 2–3 cc of  $\text{H}_2\text{O}$  and repeat the precipitation with alcohol, heating, and filtering. Add a drop of the NaOH to this alcoholic filtrate and evaporate to dryness. Dissolve this residue in a little  $\text{H}_2\text{O}$ ; acidify with  $\text{H}_2\text{SO}_4$  (1+5), using 3 or 4 drops in excess; and transfer to a small flask. Add 4 drops of the  $\text{NaNO}_2$  soln and about 5 cc of  $\text{CS}_2$ . Shake until all the I is extracted and filter off the acid soln from the  $\text{CS}_2$ . Wash the flask, filter and contents with cold  $\text{H}_2\text{O}$  and transfer the  $\text{CS}_2$  containing the I in soln to a Nessler tube, using approximately 5 cc of  $\text{CS}_2$ . In washing the filter, make the contents of the tube up to definite volume, usually 12–15 cc, and compare the color with that of other tubes containing known quantities of I dissolved in  $\text{CS}_2$ . Prepare these standard tubes by treating measured quantities of a soln of known KI content as described above, beginning with "acidify with  $\text{H}_2\text{SO}_4$  (1+5)."

Transfer separately the acid soln of the sample and the standards from which the I has been removed to small flasks. To the standards add definite measured quantities of a bromide soln of known strength, and to each of the flasks containing sample and standards add 5 cc of  $\text{CS}_2$ . Add saturated and freshly prepared Cl water, 1 cc at a time, shaking after each addition until all the Br is set free. Avoid a large excess of the Cl, as a bromo-chloride may form and spoil the color reaction. Filter off the  $\text{H}_2\text{O}$  soln from the  $\text{CS}_2$  thru a moistened filter, wash the contents of the filter

2 or 3 times with  $H_2O$ , and then transfer to a Nessler tube by means of about 1 cc of  $CS_2$ . Repeat this extraction of the filtrate twice, using 3 cc of  $CS_2$  each time. The combined  $CS_2$  extracts usually amount to 11.5–12 cc. Add enough  $CS_2$  to the tubes to bring them to a definite volume, usually 12–15 cc, and compare the sample with the standards. If, when using this method near its upper limit, the quantities of  $CS_2$  recommended do not extract all the Br, make one or two extra extractions with  $CS_2$ ; transfer the extracts to another tube; and compare the color with some of the lower standards. Add the readings thus obtained to the others.

Results closely approximating the true values for I and Br can be obtained in a shorter time on most samples by omitting the extractions with alcohol and comparing the color of the  $CS_2$  solns directly in the extraction flasks.

## ARSENIC—OFFICIAL

78

## REAGENTS AND APPARATUS

The reagents, solns, and apparatus used are described under XXIX, 1 and 2.

79

## DETERMINATION

Take a portion of the alkaline filtrate, 71, that contains not more than 0.03 mg of  $As_2O_3$ . If the quantity taken is greater than 10 cc, evaporate the soln to about that volume on a steam bath. Transfer the soln into the generator of the apparatus described under XXIX, with the aid of about 10 cc of  $H_2O$ , add 20 cc of  $H_2SO_4$  (1+2), and proceed as directed under XXIX, 4, beginning with "add 5 cc of the KI reagent."

80

## BORIC ACID—OFFICIAL

(Glassware containing boron must not be used in this determination.)

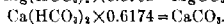
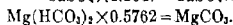
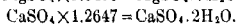
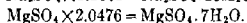
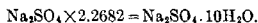
*Qualitative test.*—Evaporate to dryness a part of the alkaline filtrate under 71, treat with 1–2 cc of  $H_2O$ , and slightly acidify with HCl (1+1). Add about 25 cc of 95% alcohol, boil, filter, and repeat the extraction of the residue. Make the filtrate slightly alkaline with NaOH soln and evaporate to dryness. Add a little  $H_2O$ , slightly acidify with the dilute HCl, and place a strip of turmeric paper in the liquid. Evaporate to dryness on a steam bath and continue the heating until the turmeric paper is dry. If  $H_3BO_3$  is present, the turmeric paper takes on a cherry-red color. As a confirmatory test, apply a drop of  $NH_4OH$  (1+1) to the reddened paper. A dark olive color will be due to boric acid (cf. XXXII, 16).

*Quantitative method.*—If necessary to determine  $H_3BO_3$  quantitatively, use the Goode method.<sup>15</sup>

81 METHOD OF REPORTING RESULTS IN WATERS AND BRINE<sup>16</sup>—TENTATIVE

Report radicals and anhydrous salts in terms of mg per liter or, in the case of highly concentrated waters, in terms of g per liter. For the benefit of physicians, in the case of medicinal waters, report also the salts in terms of grains per quart, using the factor 0.014600 to convert mg per liter to grains per quart. In reporting salts in terms of grains per quart, convert the salts that have water of crystallization to the hydrated form as expressed in the U. S. Pharmacopoeia and in the National Formulary, and convert the  $Mg(HCO_3)_2$  to  $MgCO_3$  and  $Ca(HCO_3)_2$  to  $CaCO_3$ . Use the following factors in these calculations:





When a complete analysis is made report the error of analysis and state how it is distributed. Report only significant figures.

Report Fe and Al together when present in unimportant quantities and in calculations consider them as Fe. When Fe and Al are present in larger quantities, make the separation and report each separately.

In calculating the hypothetical combinations of acid and basic ions, join  $\text{NO}_3$ ,  $\text{NO}_2$ ,  $\text{BO}_3$ , and  $\text{AsO}_4$  to Na; I and Br to K; and  $\text{PO}_4$  to Ca. Assign the residual basic ions in the following order: NH, Li, K, Na, Mg, Ca, Sr, Mn, Fe, and Al, to the residual acid ions in the following order: Cl,  $\text{SO}_4$ ,  $\text{CO}_3$ , and  $\text{HCO}_3$ . In case  $\text{HCO}_3$  is not present in a sufficient quantity to join with all the Ca, the residual Ca is joined to  $\text{SiO}_2$  to form  $\text{CaSiO}_3$ , and Mn, Fe, and Al are calculated to the oxides  $\text{Mn}_2\text{O}_3$ ,  $\text{Fe}_2\text{O}_3$ , and  $\text{Al}_2\text{O}_3$ , respectively.

Use equivalent combining weights or their reciprocals in uniting the radicals, and when necessary for the purpose of comparison, in reducing salts to radicals and reuniting the radicals in the order specified above.

The equivalent combining weight of a radical is obtained by dividing its weight by its valence. The equivalent combining weight of a salt is obtained by dividing its molecular weight by the product of the valency of the basic element and the number of atoms of the basic element in the salt.

The procedure in calculating the hypothetical combinations by the use of the equivalent combining weights and their reciprocals is as follows:

Multiply the weights obtained, expressed in mg per liter, or, in the case of highly concentrated waters, in g per liter, for each radical to be combined, by the corresponding reciprocal of the equivalent combining weights. If the Na and K are to be determined by calculation, as is frequently the case, subtract the sum of the values obtained (reacting values) for the basic radicals from the sum of the reacting values for the acid radicals. The difference represents the reacting value of the undetermined Na and K. When all the constituents in the  $\text{H}_2\text{O}$  have been determined the sums of the reacting values of the acid and of the basic radicals should be very nearly the same. In this case, if the difference is reasonable and well within the limit of accuracy of the methods used, it may be distributed equally among all the radicals determined, or among those that the analyst believes to be less accurate than the others. If the difference is unreasonably great, repeat the analysis in whole or in part. The sums of the reacting values of the acid and basic radicals must be equal before proceeding with the calculation. Obtain the reacting values of the salts by subtracting in succession the reacting values of the radicals in the specified order. To convert these values to mg per liter of the respective salts multiply each of them by the equivalent combining weight of the respective salt.

*Equivalent Combining Weights and Their Reciprocals Based on  
International Atomic Weights, 1935*

NEGATIVE RADICALS	EQUIVALENT COMBINING WEIGHTS	RECIPROCAL OF EQUIVALENT COMBINING WEIGHTS	POSITIVE RADICALS	EQUIVALENT COMBINING WEIGHTS	RECIPROCAL OF EQUIVALENT COMBINING WEIGHTS
NO <sub>3</sub>	62.008	0.01613	NH <sub>4</sub>	18.0392	0.05543
BO <sub>3</sub>	42.82	0.02335	Li	6.940	0.14409
AsO <sub>4</sub>	46.30	0.02160	K	39.096	0.02558
I	126.92	0.00788	Na	22.997	0.04348
Br	79.916	0.01251	Mg	12.16	0.08224
PO <sub>4</sub>	31.673	0.03157	Ca	20.04	0.04990
HS	33.0678	0.03024	Sr	43.815	0.02282
S	16.03	0.06238	Ba	68.68	0.01456
SiO <sub>3</sub>	38.03	0.02630	Mn	27.465	0.03641
O	8.0000	0.12500	Fe <sup>II</sup>	27.92	0.03582
Cl	35.457	0.02820	Fe <sup>III</sup>	18.613	0.05372
SO <sub>4</sub>	48.03	0.02082	Al	8.99	0.11123
CO <sub>3</sub>	30.000	0.03333	Cu	31.785	0.03146
HCO <sub>3</sub>	61.0078	0.01639			

SALTS	EQUIVALENT COMBINING WEIGHTS	RECIPROCAL OF EQUIVALENT COMBINING WEIGHTS	SALTS	EQUIVALENT COMBINING WEIGHTS	RECIPROCAL OF EQUIVALENT COMBINING WEIGHTS
NH <sub>4</sub> Cl	53.4962	0.01869	MgSO <sub>4</sub>	60.19	0.01661
LiCl	42.397	0.02359	MgCO <sub>3</sub>	42.16	0.02372
Li <sub>2</sub> SO <sub>4</sub>	54.970	0.01819	Mg(HCO <sub>3</sub> ) <sub>2</sub>	73.1678	0.01367
Li <sub>2</sub> CO <sub>3</sub>	36.940	0.02707	Mg(NO <sub>3</sub> ) <sub>2</sub>	74.168	0.01348
LiHCO <sub>3</sub>	67.9478	0.01472	CaCl <sub>2</sub>	55.497	0.01802
KCl	74.553	0.01341	CaSO <sub>4</sub>	68.07	0.01469
K <sub>2</sub> SO <sub>4</sub>	87.126	0.01148	CaCO <sub>3</sub>	50.04	0.01998
K <sub>2</sub> CO <sub>3</sub>	69.096	0.01447	Ca(HCO <sub>3</sub> ) <sub>2</sub>	81.0478	0.01234
KHCO <sub>3</sub>	100.1038	0.00999	CaSiO <sub>3</sub>	58.07	0.01722
KI	166.016	0.00602	Ca <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub>	51.713	0.01934
KBr	119.012	0.00840	SrSO <sub>4</sub>	91.845	0.01089
NaCl	58.454	0.01711	SrCO <sub>3</sub>	73.815	0.01355
NaBr	102.913	0.00972	Sr(HCO <sub>3</sub> ) <sub>2</sub>	104.8228	0.00954
NaI	149.917	0.00667	BaSO <sub>4</sub>	116.71	0.00857
Na <sub>2</sub> SO <sub>4</sub>	71.027	0.01408	Ba(HCO <sub>3</sub> ) <sub>2</sub>	129.6878	0.00771
Na <sub>2</sub> CO <sub>3</sub>	52.997	0.01887	MnSO <sub>4</sub>	75.495	0.01324
NaHCO <sub>3</sub>	84.0048	0.01190	MnCO <sub>3</sub>	57.463	0.01740
NaNO <sub>2</sub>	69.005	0.01449	Mn(HCO <sub>3</sub> ) <sub>2</sub>	88.4728	0.01130
NaNO <sub>3</sub>	85.005	0.01176	FeSO <sub>4</sub>	75.95	0.01317
NaBO <sub>3</sub>	65.817	0.01519	Fe <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub>	66.643	0.01500
Na <sub>2</sub> AsO <sub>4</sub>	69.300	0.01443	FeCO <sub>3</sub>	57.92	0.01726
NaF	41.997	0.02381	Fe(HCO <sub>3</sub> ) <sub>2</sub>	88.9278	0.01124
NaHS	56.0648	0.01784	Fe <sub>2</sub> O <sub>3</sub>	26.613	0.03758
Na <sub>3</sub> PO <sub>4</sub>	54.670	0.01829	Al <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub>	57.02	0.01754
Na <sub>2</sub> S	39.027	0.02562	Al <sub>2</sub> O <sub>3</sub>	16.99	0.05886
Na <sub>2</sub> SiO <sub>3</sub>	61.027	0.01639			
MgCl <sub>2</sub>	47.617	0.02100			

## INDUSTRIAL WATER

## 83 SOLIDS IN SOLUTION—OFFICIAL

Proceed as directed under 7.

## 84 CHLORIDE—OFFICIAL

Proceed as directed under 21.

## 85 CARBONIC AND BICARBONIC ACIDS—OFFICIAL

Proceed as directed under 55.

## 86 NITRATES—OFFICIAL

Proceed as directed under 17 or 19.

## 87 SILICA—OFFICIAL

Proceed as directed under 56. Generally one evaporation with HCl for removal of  $\text{SiO}_2$  is sufficient.

## 88 IRON AND ALUMINUM—OFFICIAL

Proceed as directed under 57.

## 89 CALCIUM—OFFICIAL

If no  $\text{H}_2\text{PO}_4$  is present, concentrate the filtrate from the determination of Fe and precipitate with  $\text{NH}_4\text{OH}$  and oxalic acid as directed under 61. (Usually one precipitation is sufficient.)

## 90 MAGNESIUM—OFFICIAL

Proceed as directed under 63.

## 91 SULFURIC ACID AND ALKALIES—OFFICIAL

Proceed as directed under 64 and 66. For technical purposes sufficient accuracy is obtained by determining the acids and the bases, except Na and K, and then calculating the excess of acid over basic ions to the Na salt, stating the alkali thus found as Na and K by difference.

## 92 TEMPORARY HARDNESS—OFFICIAL

The difference between the alkalinity after boiling, 94, and the alkalinity before boiling, 93, is the temporary hardness in parts per million of  $\text{CaCO}_3$ .

ALKALINITY<sup>17</sup>

## 93 I. Before Boiling—Official

Measure 100 cc of the sample into a 250 cc white glass-stoppered bottle; add 2.5 cc of erythrosine (0.1 g of the Na salt in 1 liter of  $\text{H}_2\text{O}$ ), 5 cc of  $\text{CHCl}_3$  (neutral to erythrosine), and 0.02 N  $\text{H}_2\text{SO}_4$  in small quantities, shaking the bottle vigorously after each addition of the acid. The rose color gradually disappears and is finally discharged by 1 or 2 drops of the acid. A white paper held back of the bottle facilitates the detection of the end point. Multiply the number of cc of 0.02 N  $\text{H}_2\text{SO}_4$  used by 10 to obtain the number of p.p.m. of alkalinity in terms of  $\text{CaCO}_3$ .

## 94 II. After Boiling—Official

Boil 100 cc of the sample in a porcelain dish gently for 30 min. Cool, transfer to a 100 cc volumetric flask, and fill to the mark with recently boiled and cooled  $\text{H}_2\text{O}$ .

Filter thru a dry paper and determine the alkalinity of the filtrate as directed under 93, making the proper calculation for the aliquot used and calculating in terms of  $\text{CaCO}_3$  the parts per million of alkalinity after boiling.

95

TOTAL HARDNESS<sup>14</sup>—OFFICIAL

Add sufficient 0.05  $N$   $\text{H}_2\text{SO}_4$  to 200 cc of the sample contained in a 500 cc Pyrex or similar glass Erlenmeyer flask to neutralize the alkalinity, the quantity required being calculated from the results obtained as directed under 93. Measure 200 cc of  $\text{H}_2\text{O}$  into a similar flask. Treat the contents of each flask in the following manner: Boil 15 min. to expel free  $\text{CO}_2$ , add 25 cc of soda reagent (0.1  $N$ , equal parts of  $\text{NaOH}$  and  $\text{Na}_2\text{CO}_3$ ), boil 10 min., cool, rinse into 200 cc volumetric flasks, and dilute to 200 cc with boiled  $\text{H}_2\text{O}$ . Filter, rejecting the first 50 cc, and titrate 50 cc of each filtrate with the 0.05  $N$   $\text{H}_2\text{SO}_4$  in the presence of methyl orange or erythrosine indicator. The total hardness in p.p.m. of  $\text{CaCO}_3$  is equal to 50 times the difference between the cc of 0.05  $N$   $\text{H}_2\text{SO}_4$  used in titrating the aliquot of the blank and the aliquot of the sample.

96

## PERMANENT OR NON-CARBONATE HARDNESS—OFFICIAL

The difference between the alkalinity before boiling, 93, and the total hardness, 95, is the permanent or non-carbonate hardness expressed as p.p.m. of  $\text{CaCO}_3$ .

## IRRIGATING WATER

97

## GENERAL METHODS—OFFICIAL

Determine the solids in soln,  $\text{Cl}$ ,  $\text{CO}_3$  and  $\text{HCO}_3$ ,  $\text{Ca}$ ,  $\text{Mg}$ , and  $\text{H}_2\text{SO}_4$  as directed under 7, 21, 55, 61, 63, and 64, respectively. To make the hypothetical combination, calculate  $\text{Ca}$  and  $\text{Mg}$  to the acid ions in the following order:  $\text{HCO}_3$ ,  $\text{SO}_4$ , and  $\text{Cl}$ . Then calculate the remaining acid ions, including  $\text{CO}_3$ , to the corresponding salts of  $\text{Na}$ .

BLACK ALKALI<sup>15</sup>—OFFICIAL

98

## REAGENTS

(a) *Sodium carbonate*.—0.02  $N$ . 1 cc = 0.00106 g of  $\text{Na}_2\text{CO}_3$ .

(b) *Carbon dioxide-free water*.—Boil  $\text{H}_2\text{O}$  vigorously until approximately one-third of the original volume is evaporated, cool, and stopper.

99

## DETERMINATION

Transfer 200 cc of the sample to a Pt or Ag dish; add 50–100 cc of the  $\text{Na}_2\text{CO}_3$  soln, according to the quantity of soluble salts of  $\text{Ca}$  and  $\text{Mg}$  present; and evaporate to dryness. Rub up the residue with  $\text{CO}_2$ -free  $\text{H}_2\text{O}$  and transfer to a 100 cc volumetric flask. (An ordinary laboratory wash bottle should not be used to transfer the residue, as the  $\text{CO}_2$  from the breath of the operator will vitiate the results.) Dilute to the mark, shake thoroly, and allow to stand until clear (12–15 hours). Remove 50 cc of the clear supernatant liquid, equivalent to half of the original quantity of sample and  $\text{Na}_2\text{CO}_3$  added, and transfer to a 250 cc glass-stoppered flask or a stoppered titration bottle of clear glass, without any tinge of pink. Add 5 cc of  $\text{CHCl}_3$ , neutral to erythrosine, and 1 cc of erythrosine indicator (0.1 g of the  $\text{Na}$  salt in 1 liter of  $\text{H}_2\text{O}$ ) and titrate with 0.02  $N$   $\text{H}_2\text{SO}_4$  until the color disappears. Shake the soln vigorously after each addition of the acid. The milky appearance produced by the  $\text{CHCl}_3$  makes the reading of the end point sharp and certain.

(1) If less  $\text{H}_2\text{SO}_4$  is required than is equivalent to half of the  $\text{Na}_2\text{CO}_3$  added, due to some of the  $\text{Na}_2\text{CO}_3$  reacting with soluble salts of Ca and Mg, the soln originally contained no black alkali in excess, but rather an excess of the so-called permanent or non-carbonate hardness. Express the hardness in terms of  $\text{CaCO}_3$  or  $\text{CaSO}_4$ . With irrigating waters the latter form is better. The difference between the number of cc of the  $\text{H}_2\text{SO}_4$  required and half the number of cc of the  $\text{Na}_2\text{CO}_3$  added  $\times$  the factor 0.00136 = the equivalent of  $\text{CaSO}_4$  in 100 cc of the sample.

(2) If more  $\text{H}_2\text{SO}_4$  is required than that equivalent to half of the  $\text{Na}_2\text{CO}_3$  added, black alkali was originally present in the soln, and the difference in cc  $\times$  the factor 0.00106 = the black alkali in terms of  $\text{Na}_2\text{CO}_3$  in 100 cc of sample.

### BRINE

#### IODIDE IN THE PRESENCE OF CHLORIDE AND BROMIDE

##### *Method I.<sup>20</sup>—Tentative*

#### 100

##### REAGENTS

(a) *Sodium hydroxide-sodium carbonate soln.*—Dissolve 50 g of a mixture of equal weights of NaOH and  $\text{Na}_2\text{CO}_3$  in  $\text{H}_2\text{O}$  and dilute to 1 liter.

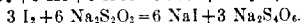
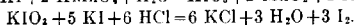
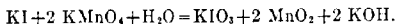
(b) *Sodium hydroxide soln.*—Dissolve 4 g of NaOH in  $\text{H}_2\text{O}$  and dilute to 100 cc.

(c) *Potassium permanganate soln.*—Dissolve 50 g of  $\text{KMnO}_4$  in  $\text{H}_2\text{O}$  and dilute to 1 liter.

(d) *Sodium thiosulfate soln.*—0.05 N. Dissolve 12.4 g of recrystallized  $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$  in  $\text{H}_2\text{O}$  and dilute to 1 liter.

#### 101

##### REACTIONS



#### 102

##### DETERMINATION

Take a quantity of the brine that contains not more than 0.1 g of I nor more than 10 g of total salts. Adjust the volume to 100–150 cc, and add a sufficient quantity of Reagent (a) to precipitate the Ca and Mg. Boil, filter off the precipitate of Ca and Mg, and wash with hot  $\text{H}_2\text{O}$ . Introduce the filtrate into an Erlenmeyer flask, adjust the volume to about 100 cc, neutralize with  $\text{H}_2\text{SO}_4$  (1+9), and add 1 cc of the NaOH soln. Heat to boiling; add an excess of about 0.5 cc of the  $\text{KMnO}_4$ ; continue heating until the precipitate begins to coagulate; and allow to cool. Add sufficient 95% alcohol or  $\text{H}_2\text{O}_2$  to bleach the permanganate color and set the beaker on a steam bath. When the precipitate has settled, filter and wash with hot  $\text{H}_2\text{O}$ . After cooling, add 1–2 g of KI, acidify with HCl, and titrate with the 0.05 N thiosulfate soln. Since one-sixth of the I titrated represents the quantity originally present, 1 cc of 0.05 N  $\text{Na}_2\text{S}_2\text{O}_3$  soln = 1.058 mg of I.

##### *Method II.<sup>21</sup>—Tentative*

#### 103

##### REAGENTS

(a) *Potassium iodide soln.*—Dissolve 200 g of KI, free from iodate, in  $\text{H}_2\text{O}$  and dilute to 1 liter.

(b) *Starch iodide paper.*—Dip strips of filter paper in 25 cc of the starch indicator, VI, 3(e), that has been mixed with 5 cc of a 10% soln of KI.

104

## APPARATUS

(a) *Reaction flask*.—A glass-stoppered flask of 200–400 cc capacity provided with inlet and outlet tubes, the inlet tube having a stopcock and reaching nearly to the bottom of the flask and the outlet tube having a bulb of about 25 cc capacity blown near the center to lessen the danger of the absorbing soln being drawn back into the reaction flask.

(b) *Tall absorption bottle*.

105

## DETERMINATION

Reduce by evaporation a quantity of the sample that contains not more than 0.1 g of I to a volume of 25 cc, and place in the glass-stoppered reaction flask. Add 5 cc of HCl (1+1), insert the stopper, and then add thru the inlet tube 50 cc of freshly prepared Cl water. Place the end of the outlet tube in the tall absorption flask in which is placed 35 cc of 10% soln of  $K_2CO_3$  diluted to 150 cc. Heat the reaction flask and boil gently until most of the Cl and Br has been distilled into the alkali. Connect the inlet tube to a  $CO_2$  generator and complete the distillation by simultaneous boiling and bubbling of  $CO_2$  thru the sample. Continue for 10 min., testing for the presence of Cl and Br by holding a piece of starch iodide paper at the end of the outlet tube. Remove the source of heat and bubble  $CO_2$  thru the apparatus until it is cool. Add 5 cc of the soln of KI and titrate the liberated I with 0.05 N  $Na_2S_2O_3$  soln, 100 (d).

BROMIDE IN THE PRESENCE OF CHLORIDE BUT NOT IODIDE<sup>22</sup>—TENTATIVE

106

## REAGENT

*Alkaline sodium sulfite soln*.—Dissolve 4 g of  $Na_2SO_3$  and 0.8 g of  $Na_2CO_3$  in  $H_2O$  and dilute to 100 cc.

107

## APPARATUS

(a) *Reaction cylinder*.

(b) *Two high-form Drechsel gas washing bottles*.

Join the reacting cylinder and the two gas washing bottles as shown in Fig. 45.

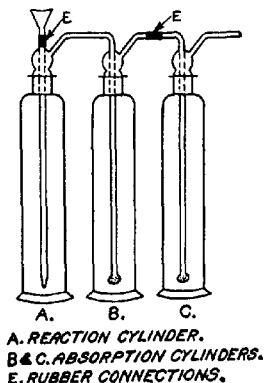
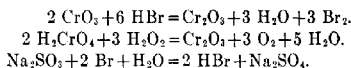


FIG. 45.—REACTION CYLINDER TO BE USED IN THE DETERMINATION OF BROMIDE

108

## REACTIONS



109

## DETERMINATION

Take a quantity of the brine that contains not more than 10 g of total salts. (The sample should not be too acid.) Evaporate to dryness or nearly so. Charge reaction cylinder A by introducing glass beads to a depth of about 1 inch, followed by 15 g of  $\text{CrO}_3$  crystals and finally enough glass beads to fill the cylinder half full. Add 20 cc of alkaline  $\text{Na}_2\text{SO}_3$  soln, 106, to the first absorption cylinder and 5 cc to the second. Dilute each to about 200 cc. Connect the 3 cylinders and draw a current of air thru slowly. Wash the sample into the reaction cylinder with sufficient  $\text{H}_2\text{O}$  to make about 25 cc of soln. Aspirate until the contents of the reaction cylinder are in soln and thoroly mixed, close the inlet tube with a small piece of rubber tubing and clamp, and reduce the pressure in the apparatus slightly by suction in order to guard against any possible escape of Br at the ground-glass stopper. Allow to stand overnight and then aspirate with a rather strong current of air (about 0.5–0.75 liter per min.) for 3 hours, adding four 2 cc portions of 3%  $\text{H}_2\text{O}_2$  soln to the reaction flask at 30 min. intervals. Stop the aspiration and evaporate the contents of the 2 absorption cylinders nearly to dryness. Clean out the reaction cylinder and freshly charge with glass beads and 15 g of  $\text{CrO}_3$  crystals. To the first absorption cylinder add 10 g of KI crystals dissolved in 200 cc of  $\text{H}_2\text{O}$  and to the second, 3 or 4 g in a like quantity of  $\text{H}_2\text{O}$ . Connect the apparatus, draw thru a slow current of air, and transfer the contents of the evaporating dish to the reaction cylinder by means of the small funnel, using 25 cc of  $\text{H}_2\text{O}$ . Aspirate until all the Br is evolved (about 1 hour) and titrate the KI soln with standard 0.05 N  $\text{Na}_2\text{S}_2\text{O}_3$  soln, 100(d). 1 cc of  $\text{Na}_2\text{S}_2\text{O}_3$  = 3.996 mg of Br.

BROMIDE IN THE PRESENCE OF CHLORIDE AND IODIDE<sup>a</sup>—TENTATIVE

(Collaborative work indicates that the following is the best method that has been published for the determination of Br in the presence of Cl and I, but the results obtained show that only about 95% of the Br present is recovered when 80 mg of Br is contained in the portion of the sample taken for analysis. The method is satisfactory in the absence of I.)

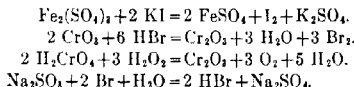
110

## REAGENT AND APPARATUS

The reagent and the apparatus and manner of connecting are described under 106 and 107.

111

## REACTIONS



112

## DETERMINATION

Introduce 10 cc of the sample into a distillation flask, adjust the volume to about 75 cc, and add 1.5–2.0 g of  $\text{Fe}_2(\text{SO}_4)_3 \cdot 9\text{H}_2\text{O}$ . Distil off the liberated I with steam, discarding the distillate. Transfer the residue from the distillation flask to a beaker,

heat to boiling, add a few drops of methyl orange, and precipitate the Fe with  $\text{NH}_4\text{OH}$ , avoiding an excess of  $\text{NH}_4\text{OH}$ , as a precipitate of  $\text{Ca}(\text{OH})_2$  is bulky and difficult to wash. Filter off the  $\text{Fe}(\text{OH})_3$ , wash with hot  $\text{H}_2\text{O}$ , and evaporate the filtrate and washings to dryness or nearly so, taking care that during the evaporation the soln does not become acid from hydrolysis of  $\text{MgCl}_2$ . From this point proceed as directed under 109, beginning with "Charge reaction cylinder."

#### SALT<sup>24</sup>

113

##### PREPARATION OF SAMPLE—TENTATIVE

If the sample is coarser than 20 mesh, grind so that all will pass thru a 20-mesh sieve, but avoid undue grinding so that as much as possible will be retained on an 80-mesh sieve. Mix sample by quartering and weigh all needed portions as nearly at the same time as possible.

114

##### MOISTURE—TENTATIVE

Place about 10 g of the sample in a dry, weighed Erlenmeyer flask of about 200 cc capacity. Weigh the flask and sample. Spread the sample evenly over the bottom of the flask by shaking gently and insert a small funnel in the neck. Heat the flask and sample for periods of 1 hour each on a triangle over the low, open flame of a gas stove at a temp. of about  $250^\circ$  until 2 consecutive weighings agree within 5 mg. Shake the flask occasionally so that the sample will dry evenly. Report the loss of weight as moisture.

115

##### MATTERS INSOLUBLE IN WATER—TENTATIVE

Place 10 g of the sample in a 250 cc beaker, add 200 cc of  $\text{H}_2\text{O}$  at room temp., and let stand 30 min., stirring frequently. Filter thru a weighed Gooch crucible with asbestos mat dried at  $110^\circ$ . Transfer the residue to a Gooch crucible with the aid of a rubber-tipped glass rod, using a total of not more than 50 cc of  $\text{H}_2\text{O}$ . Wash the residue with small portions of  $\text{H}_2\text{O}$ , about 10 portions of 10 cc each, until 10 cc of the filtrate shows only a faint opalescence upon addition of a few drops of  $\text{AgNO}_3$  soln. Dry crucible and contents to constant weight at  $110^\circ$ . Call increase in weight of Gooch crucible, "matters insoluble in  $\text{H}_2\text{O}$ ," and report results in percentage on a moisture-free basis. If matters insoluble in  $\text{H}_2\text{O}$  exceed 0.1%, determine their nature.

116

##### MATTERS INSOLUBLE IN ACID<sup>25</sup>—TENTATIVE

Treat 10 g of the sample with 200 cc of  $\text{HCl}$  (1+19), boil 2–3 min., and let stand 30 min., stirring frequently. Filter thru a Gooch crucible with mat, dried at  $110^\circ$ . Express results in percentage.

##### 117 PREPARATION OF SOLUTION FOR SULFATE, CALCIUM, AND MAGNESIUM—TENTATIVE

Weigh about 20 g of the sample, transfer to a 400 cc beaker, and dissolve in 200 cc of  $\text{HCl}$  (1+3). Cover beaker, heat to boiling, and continue boiling gently for 10 min. Filter thru a paper filter and wash residue with small quantities of hot  $\text{H}_2\text{O}$  until the filtrate is free from chlorides. Unite the filtrate and washings, cool, and make to a volume of 500 cc (soln A).

118

##### SULFATE—TENTATIVE

Place 250 cc of soln A, 117, in a 400 cc beaker of resistant glass, heat to boiling, and add a slight excess of a hot 10%  $\text{BaCl}_2$  soln dropwise while stirring. Concen-



trate by heating gently and finally evaporate to dryness on a steam bath. Facilitate removal of free acid by stirring the partly dry residue. Wash the precipitate by decantation with small quantities of hot  $\text{H}_2\text{O}$ , finally transferring the precipitate to a close grained filter paper with the aid of a rubber-tipped glass rod and stream of hot  $\text{H}_2\text{O}$ . Wash the precipitate on the filter until the filtrate is free from chlorides. Test the filtrate for the presence of Ba. Dry and ignite the filter and precipitate over a Bunsen flame. Report the percentage of  $\text{SO}_4$  in the sample on a moisture-free basis.

## 119

## CALCIUM—TENTATIVE

Place the remainder of soln A in a 400 cc beaker of resistant glass. Add an excess of 10% oxalic acid soln (10 cc usually will be sufficient). Add a few drops of methyl orange indicator and neutralize while hot by adding  $\text{NH}_4\text{OH}$  dropwise, stirring constantly. Add about 1 cc excess of the  $\text{NH}_4\text{OH}$ , stir, and let stand in a warm place for 3 hours. Decant the supernatant liquid thru a filter, reserving the filtrate for the determination of Mg. Test the filtrate for Ca with  $\text{NH}_4$  oxalate soln. Wash the precipitate in a beaker once with 10 cc of a 1%  $\text{NH}_4$  oxalate soln, decanting thru filter paper. Combine filtrate and washings. Dissolve the precipitate on a filter with hot  $\text{HCl}$  (1+1), using the same beaker, dilute to 100 cc, add a little more oxalic acid, and precipitate as before. After standing 3 hours, filter and wash with the  $\text{NH}_4$  oxalate soln as before, reserving the filtrate and washings. Transfer the precipitate to a crucible, dry, ignite, and heat over a blast lamp to constant weight. Report as percentage of Ca on a moisture-free basis.

## 120

## MAGNESIUM—TENTATIVE

Combine the filtrates and washings from the Ca determination, concentrate if necessary by boiling gently to a volume of about 150 cc, and proceed as directed under 63. Report as percentage of Mg on a moisture-free basis.

## 121

## METHOD OF REPORTING RESULTS IN SALT—TENTATIVE

(In the absence of added drying agents such as  $\text{MgCO}_3$ , Ca phosphate, etc.)

Convert the sulfate to  $\text{CaSO}_4$  and the unused Ca to  $\text{CaCl}_2$ , unless the sulfate in the sample exceeds the quantity necessary to combine with the Ca, in which case convert the Ca to  $\text{CaSO}_4$  and the unused sulfate first to  $\text{MgSO}_4$  and the remaining sulfate, if any, to  $\text{Na}_2\text{SO}_4$ . Convert the unused Mg to  $\text{MgCl}_2$ . Add the percentages of  $\text{CaCl}_2$  and  $\text{MgCl}_2$ . Report on a moisture-free basis the percentage of matters insoluble in  $\text{H}_2\text{O}$ , of sulfate, of Ca, of Mg, of  $\text{CaSO}_4$ , and of  $\text{CaCl}_2$  and  $\text{MgCl}_2$ . Report also the results of the qualitative examination of matters insoluble in  $\text{H}_2\text{O}$ , if the quantity exceeds 0.1% on a moisture-free basis.

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## XXXVIII. RADIOACTIVITY

1

### QUALITATIVE TEST—OFFICIAL

(Applicable to solids.)

Charge the alpha ray electroscope thru the charging wire (10) to bring the leaf to a suitable position on the scale in the microscope (9). Close the door (12) and record the position of the leaf on the scale at frequent intervals, until the rate of fall of the leaf is constant. Calculate the rate of fall of the leaf in divisions per min., designating the figure obtained as the natural leak of the instrument for that particular determination.

Place a convenient portion of the sample on the pan (11) and introduce it into the discharge chamber, close the door, recharge the leaf system thru the charging wire (10), and record the rate of fall of the leaf in divisions per min. over the same range of scale as before, until the rate becomes constant, recharging if necessary. A rate of fall in excess of the natural leak of the instrument shows that the sample is radioactive.

### QUANTITATIVE METHODS

#### *Emanation or radon method*<sup>1</sup>—Official

2

#### REAGENT

*Sodium hydroxide soln.*—Dissolve 60 g of NaOH in H<sub>2</sub>O, dilute to 3 liters, boil the soln at least 10 min., and keep hot until ready for use. Prepare the soln the day it is to be used.

3

#### APPARATUS

(a) *Gas collection apparatus.*—Two flat-bottomed long-necked Florence flasks, the one containing the sample having a capacity of 0.2–2 liters and the other a capacity of 2 liters (Nos. 1 and 2 in Fig. 46); 2 overflow flasks of about 300 cc capacity (3, 4); 1 gas-collecting buret 4 cm in diameter and 50 cm in length (5); 2 gas burners; and 2 iron supports provided with iron clamps, 1 iron tripod, sufficient rubber and glass tubing, wire gauzes with asbestos centers, and pinch cocks. Connect the apparatus as shown in Fig. 46.

(b) *Gas transfer apparatus.*—One gas transfer buret 4 cm in diameter and 50 cm in length (6); 1 separatory funnel or leveling bulb with a capacity of 1 liter; 2 iron supports provided with iron clamps; rubber tubing, and pinch cocks.

(c) *Calcium chloride drying tube* (7).—A 1-hole rubber stopper and suitable glass and rubber tubing.

(d) *Control tube.*—A glass vial about 2 cm in diameter and about 6½ cm long (8), a 2-holed rubber stopper, and 2 glass tubes.

(e) *Alpha ray electroscope.*—A head, which is either permanently attached, as in Fig. 46, or detachable, as furnished by laboratory supply houses; an open discharge chamber; and a microscope (9) on a permanently attached base. The microscope contains a micrometer scale. The head of the electroscope contains a leaf system including the charging wire (10), and the open discharge chamber, with a door (12), contains an adjustable support for the pan (11).

(f) *Emanation type electroscope.*—A head, either permanent or detachable, Fig. 46, a gas-tight emanation chamber (13) provided with stop cocks (16 and 17) and a microscope (14) on a permanently attached base. The microscope contains a

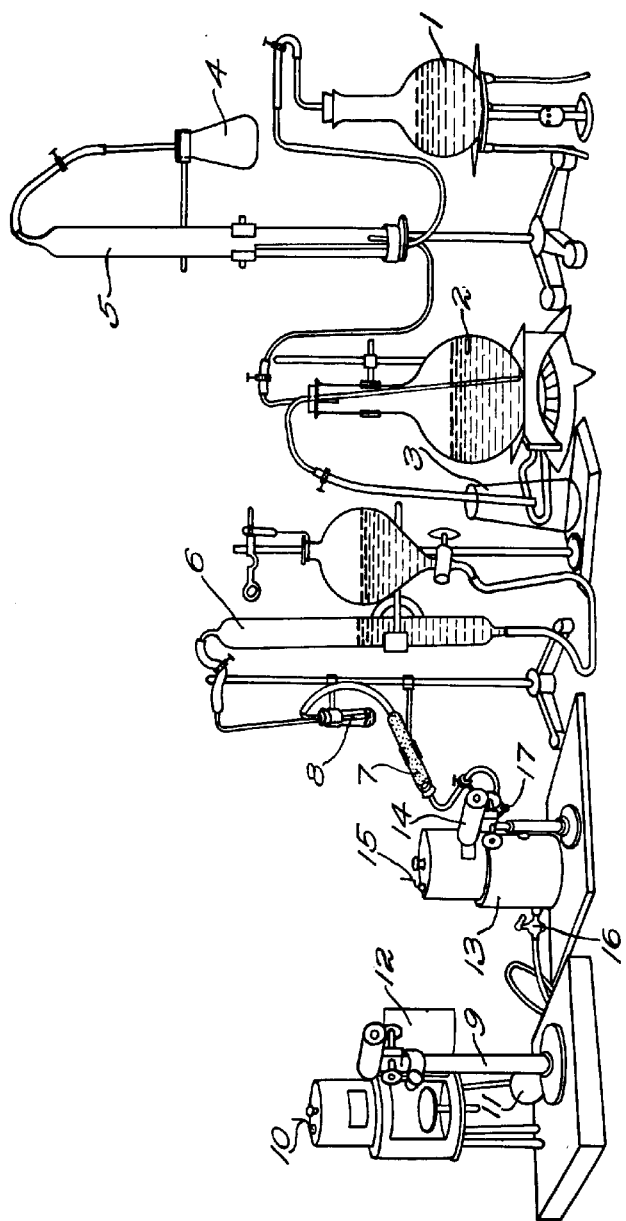


FIG. 46.—APPARATUS FOR DETERMINING RADIOACTIVITY BY THE EMANATION METHOD

micrometer scale. The head of the electroscope contains a leaf system and a charging wire (15).

(g) *Charging device*.—Or a hard rubber rod and catskin.

(h) *Stop watch*.

(i) *Vacuum pump*.

## 4

## PREPARATION OF SAMPLE

(All reagents must be free from radium and radon.)

*A. Samples completely soluble in acids:*

(1) *Solid or semi-solid form*.—Add 50 cc of  $\text{HNO}_3$  (1+9) and boil for several min. If a residue remains, add 50 cc of  $\text{HCl}$  (1+9) and again boil. (This treatment should not be applied to samples containing grease, such as face creams, the physical appearance of which will indicate that they are insoluble in aqueous solns.)

(2) *Liquid form (clear liquid, turbid liquid, or liquid containing suspended matter)*.—Add 50 cc of  $\text{HNO}_3$  (1+9) to from 1 to 10 cc of sample, boil for several min., and examine carefully for opalescence. If a portion of the sample remains undissolved, add 50 cc of  $\text{HCl}$  (1+9) and again boil. The addition of  $\text{HCl}$  to  $\text{HNO}_3$  mixtures in this case and in the case of samples in solid and semi-solid form should be avoided if possible.

If clear and limpid solns are obtained by the above procedures, take a quantity of the well-mixed sample that will produce an accurately measurable increase in the rate of fall of the leaf, dissolve as directed under (2), preferably in a Florence flask of 300 cc capacity, and proceed as directed under (c) "*Final preparation of clear solns.*"

*B. Samples wholly or partly insoluble in acids:*

(a) *Preliminary treatment:*

(1) *Solids*.—If the sample is not in powder form, grind to a fine powder, ignite a weighed portion in a porcelain dish in a muffle at dull red heat, avoiding fusion, and proceed as described below under (b) "*Treatment of ash.*"

(2) *Semi-solids*.—Ignite quite rapidly in a muffle a weighed portion of the sample contained in a porcelain dish, avoiding fusion. Heating too slowly or heating in the open may cause the sample to "creep" over the edge of the dish. Proceed as directed under (b) "*Treatment of ash.*"

(3) *Liquids immiscible with  $\text{H}_2\text{O}$* .—Evaporate a weighted or measured portion of the sample to dryness, or as nearly so as possible, on a steam bath, and dry carefully on a hot plate. Ignite the residue in a muffle, avoiding fusion. Proceed as directed under (b) "*Treatment of ash.*"

(4) *Liquids containing material which is insoluble in  $\text{HNO}_3$  (1+9)*.—Digest the sample or a suitable portion of it with  $\text{HNO}_3$  (1+9). Filter into a 300 cc Florence flask, and wash the residue thoroly with hot  $\text{H}_2\text{O}$ . Proceed as directed under "*Treatment of ash,*" beginning "Ignite the washed residue in a Pt dish . . ."

(b) *Treatment of ash:*

(1) Digest the ash obtained under (a) with  $\text{HNO}_3$  (1+9) on a steam bath. Note the quantity of acid used in this and subsequent operations so that the final clear soln can be adjusted to contain about 10% of acid by volume. Filter into a Florence flask and wash thoroly with hot  $\text{H}_2\text{O}$ . (Ordinarily a flask of 300 cc capacity is the most suitable, even if it is necessary to concentrate the filtrates by boiling.) Ignite the washed residue in a Pt dish and cover the residue with a few cc of  $\text{H}_2\text{O}$  and 5-10 cc of  $\text{HF}$ . Evaporate to dryness on a steam bath. Add  $\text{H}_2\text{O}$  and a few cc of  $\text{HNO}_3$  (1+9), digest on a steam bath, filter into a Florence flask, and wash with  $\text{H}_2\text{O}$ . Ash

the filter paper in a Pt dish and add 5-10 cc of  $H_2O$  and 1 cc of  $HNO_3$ . Examine carefully for any insoluble material, and if none is found, add the soln directly to the Florence flask, rinsing out the Pt dish several times with  $H_2O$  and adding the washings to the flask. Then follow the procedure under c "*Final preparation of clear solns.*"

(2) If an insoluble residue that does not contain  $BaSO_4$  remains, proceed as follows: Ignite the insoluble residue in a Pt dish and fuse with 5 to 10 times its weight of a fusion mixture consisting of equal weights of  $K_2CO_3$  and anhydrous  $Na_2CO_3$ . Cool, and cover with a cover-glass. Neutralize the fused mass with  $HNO_3$  (1+9) using a drop of phenolphthalein soln to note when the soln is acid. Heat on a steam bath, add a few cc excess of the  $HNO_3$ , and boil carefully. Filter the soln into the Florence flask and wash thoroly. Ignite the insoluble residue in a Pt dish and proceed as directed under "*Treatment of ash (b),*" beginning with "cover the residue with a few cc of  $H_2O$  and 5-10 cc of  $HF$ ."

(3) If the insoluble residue does contain appreciable quantities of  $BaSO_4$ , proceed as follows: Ignite the insoluble residue in a Pt crucible, mix, and fuse with 5-10 times its weight of a fusion mixture consisting of equal weights of  $K_2CO_3$  and anhydrous  $Na_2CO_3$ . Cool, boil the residue with a little  $H_2O$  until thoroly disintegrated, and filter into a separate Florence flask. Since this soln contains  $SO_4$ , do not mix with the acid filtrate obtained under (b) (1). Wash the residue with hot, normal  $Na_2CO_3$  soln until the filtrate gives no test for  $SO_4$ , and then with a little  $H_2O$ . Dissolve the washed residue ( $Ba-RaCO_3$ ) carefully with  $HNO_3$  (1+1) and wash thoroly with  $H_2O$  into a separate Florence flask. If an insoluble residue remains, proceed as directed under (b) (1), "Ignite the washed residue," etc. Combine this filtrate with the original acid filtrates.

(c) *Final preparation of clear solns:*

Boil the clear acid solns obtained under (a) or (b) for 20 min. Add dilute  $HNO_3$  to make the volume of the soln about 80% of the capacity of the flask. (The final soln should contain at least 10% by volume of acid.) Dilute with normal  $Na_2CO_3$  any alkaline solns. Cool, and seal the flask without delay, noting exact time of sealing, in the following manner: Bend a piece of glass tubing at right angles, draw out one end, and seal off. (The sealed-off arm should be about 15 cm long and the other arm about 7 cm long.) Place the short arm in a one-hole rubber stopper, so that the end is flush with bottom of stopper. Place stopper and tubing in neck of flask so that a tight fit is obtained and tie down to the flask with a cord. Examine the soln the next day. If an opalescence or precipitate is noted, filter the soln, wash the insoluble residue with hot  $H_2O$ , and treat it as described under (b) (2).

## 5

## DILUTE RADIUM SOLUTION

Measure accurately a quantity of clear standard stock soln of radium that will contain about 5 millimicrograms of radium and place it in a flat-bottomed Erlenmeyer or Florence flask of about 200 cc capacity, provided with a sealed-in glass tube extending horizontally about 12 cm. If the volume of the standard soln taken is less than 80 cc, make it up to about 160 cc with a boiled soln of  $HNO_3$  (1+12) containing about 0.1 g of  $Ba(NO_3)_2$  per 100 cc. Record the exact quantity of standard soln taken and its temp. Boil the soln rather vigorously for 20 min., carefully avoiding mechanical loss, allow to cool slightly, replace the soln lost by evaporation with an equal quantity of boiled  $H_2O$ , and draw out and seal the end of the glass tube, recording the exact time of sealing. Allow the soln to stand at least 4 days.

## 6

## STANDARDIZATION OF ELECTROSCOPE

Determine the natural leak of the emanation type electroscope as follows: Create a vacuum in the emanation chamber (13) by means of the vacuum pump. Connect the closed emanation chamber (stopcock 17) with the freshly filled  $\text{CaCl}_2$  drying tube (7) and with the glass vial (8), which should contain about 2 cc of  $\text{H}_2\text{O}$ . Allow dried air to enter the emanation chamber at the rate of one or two bubbles per second. When equilibrium is established, close off the emanation chamber by turning the stopcock (17), and illuminate the leaf system and the micrometer scale in the microscope (14) by placing a small electric bulb behind the electroscope at such a distance that the electroscope will not be heated but the light will give a good illumination on the micrometer scale. Charge the electroscope thru the charging wire (15) so as to bring the leaf in view on a suitable part of the scale. Revolve the charging wire and ground it on the inner wall of the head of the electroscope. After about 15–30 min., begin recording the exact positions of the leaf with respect to the scale, at intervals of about 10 min. for approximately an hour, noting the exact time and estimating to tenths of divisions. Calculate the average rate of fall of the leaf in divisions per min. and designate the figure obtained as the natural leak of the instrument for the particular determination.

Place about  $1\frac{1}{4}$  liters of the  $\text{NaOH}$  soln in the 2 liter flask (2) and boil vigorously for at least 10 min. Lower the flame, manipulate the clips so as to fill the gas-collecting buret (5) with the soln, and continue boiling gently. Connect the flask containing the standard  $\text{Ra}$  soln with the buret. Heat the sample gently and before excessive pressure is generated break off the capillary end inside the rubber connection, noting the exact time of breaking. Open the clip to permit evolution of gas into the buret. Continue heating the  $\text{NaOH}$  soln in the 2 liter flask (2) and boil the standard  $\text{Ra}$  soln rather vigorously for 20 min. Extinguish the flame under the sample and at the same time close the clip so as to retain the gases in the buret, and disconnect the flask containing the standard  $\text{Ra}$  soln. Before the soln is cold, draw out and seal off the glass tube of the flask. Fill the gas transfer buret (6) with the hot  $\text{NaOH}$  soln that has been boiled for at least 10 min., and connect it with the upper end of the gas-collecting buret (5), so manipulating the clips as to transfer, without loss, the collected gases from the gas-collecting buret (5) to the transfer buret (6). Shut off the burner, disconnect the transfer buret from the gas-collecting buret, and connect it with the glass vial (8), the  $\text{CaCl}_2$  drying tube (7), and the emanation chamber (13), in which a vacuum has been created. Perform this operation without loss of the collected gases. Allow these gases to pass into the emanation chamber at the rate of 1–2 bubbles per second. As soon as the  $\text{NaOH}$  soln appears at the inlet tube of the glass vial, disconnect the gas transfer buret and allow the inflow of air to continue at the same rate until bubbling ceases. Close the stopcock (17) and disconnect the glass vial and the  $\text{CaCl}_2$  drying tube. Allow the electroscope to stand  $2\frac{1}{4}$  hours, charge it, and keep it charged for 15 min.; then begin a series of readings with the aid of a stop-watch on the same range of scale used in determining the natural leak of the electroscope. After the readings have been completed, draw air thru the emanation chamber for about 10 min. by means of the vacuum pump until the radon is removed. Subtract the natural leak of the instrument in terms of divisions per min. from the rate of fall in divisions per min. when the radon was in the electroscope. Divide the number obtained into the number of millimicrocuries of radon in the standard  $\text{Ra}$  soln. In a sample sealed for less than 30 days, calculate the radon content of the sample from a table of decay or growth of radon. In a sample sealed for 30 days or more, the radon content will be equivalent to the  $\text{Ra}$

content. The quotient will be the quantity of Ra that will cause an acceleration of one division per min. in the rate of fall of the leaf. Repeat the standardization occasionally, using other dilute Ra solns prepared from the standard stock Ra soln.

## 7

## DETERMINATION

Determine the natural leak of the electroscopes, boil off the emanation from the sample, and determine its effect on the rate of fall of the leaf exactly as described previously, 6, taking the precaution of allowing the collected gases to remain in the gas-collecting or gas-transfer burets at least 10 min. for decay of any thoron that may be present. Subtract the natural leak in divisions per min. from the increased rate of fall, expressed in divisions per min., the difference being the rate of fall of the leaf due to radon in the sample. Multiply the figure obtained by the number of millimicrograms of Ra that will cause an increase of one division per min. If the sample has been allowed to stand 30 days, the result will be the quantity of Ra in the sample. If the sample has stood for less than 30 days, calculate the Ra content from tables of decay and growth of radon. If the original sample is a soln, report the content of Ra in millimicrograms per cc or per liter. If the original sample is a solid, report the content of Ra in terms of millimicrograms per g or per 100 g.

*Gamma ray method<sup>2</sup>—Tentative*

## 8

## APPARATUS

A cylindrical zinc chamber (1) of about 1000 cc capacity, which is hermetically sealed. The axis of the cylinder is vertical. On the inside is the Wulf two-fiber system (3), which is fastened to an amber insulator, and which can be charged with the aid of a charging-rod (4). The rate of movement of the fibers is determined by means of a microscope (2).

## 9

## PREPARATION OF SAMPLE

Use the whole sample or one or more subdivisions, depending upon the content of radioactivity, but do not open the individual containers. Seal any loose material in a suitable container such as a test tube.

## 10

## STANDARDS

Use known quantities of radium measured by the National Bureau of Standards.

## 11

## STANDARDIZATION OF ELECTROSCOPE

(a) *Natural leak.*—Charge the electroscopes through the charging rod by means of a charging device, 3(g), to bring one of the fibers to a suitable point on the properly illuminated microscope scale after 4, Fig. 47, is grounded—for example, at the 50 division mark or above. As the natural leak of the electroscopes in a room free from radium is very small, use a radium standard to adjust the fiber approximately to the desired division mark. Remove the standard from the room and record the time when the fiber crosses the exact division mark. Allow the electroscopes to remain charged overnight. Again record the time when the fiber crosses an exact division mark. Calculate the rate of travel of the fiber in seconds per division and designate the figure obtained as the natural leak (R) of the electroscopes for the particular determination.

(b) *Constant.*—Place a suitable radium standard containing 10–1000 micrograms of radium at an exact measured distance from the center of the electroscopes. Charge the electroscopes and record the average time, measured by a stop-watch,



for at least 6 trials, of the fiber to travel over that part of the scale used in obtaining the natural leak. Calculate the rate of travel in seconds per division and the corrected time (T) due to radium alone by the following formula:

(1)  $T = AR/R - A$ , in which A = observed time and R = natural leak.

Then calculate the constant (K) by the following formula:

(2)  $K = ST/(D)^2$ , in which S = micrograms of radium in the standard; T = corrected time found in (1); and D = distance between the center of the electroscope and the standard.

To obtain a reliable average figure for this constant, calculate K, placing the radium standard at different distances from the center of the electroscope. Use several different standards of known radium content.

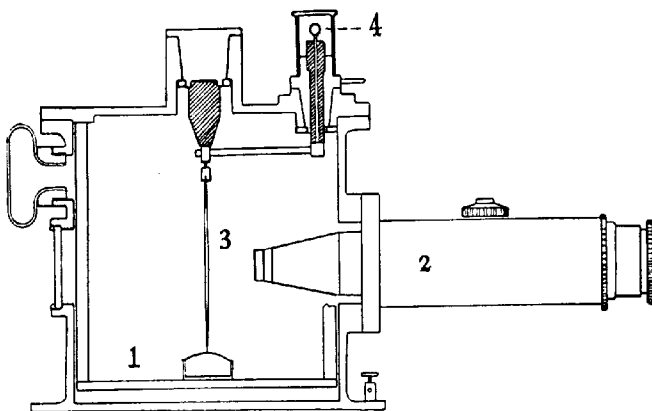


FIG. 47—GAMMA RAY ELECTROSCOPE

## 12

### DETERMINATION

Place the sample at a suitable distance from the center of the electroscope. Charge the electroscope as directed above, using if convenient a radium standard to adjust the fiber. Record the average time taken by the fiber to travel between exact division marks over all or a major portion of that part of the scale used for the standardization. If the sample contains sufficient radioactivity to permit, take average readings when it is placed at different distances from the center of the electroscope; if it contains only a relatively small quantity of radioactivity, fasten it with rubber bands to the circumference of the electroscope so as to obtain the maximum ionization. Calculate the micrograms of radium (S') or its equivalent in terms of radium by the following formula:

(3)  $S' = K(D')^2/T'$ , in which K = constant of electroscope; D' = exact distance between the center of the electroscope and center of the sample; and T' = corrected time in seconds per division due to the radioactivity only in the sample.

### SELECTED REFERENCES

- <sup>1</sup> J. Assoc. Official Agr. Chem., **8**, 531 (1925); **10**, 363 (1927); **13**, 308 (1930); **14**, 85, 309 (1931); **15**, 404 (1932); **19**, 102 (1936).
- <sup>2</sup> Ibid., **19**, 101 (1936).

## XXXIX. DRUGS

### SAMPLING—TENTATIVE

1

#### *I. Tablets and Pills*

(a) *Bulk lots*.—Mix the lot as thoroly as possible without mutilating the contents. Count, weigh, and powder thoroly at least 100 units. Calculate the average weight per unit.

(b) *Containers of 1000 or more units*.—Open and cautiously mix the entire contents without mutilation and divide into 2 parts, one of which is liberal for analysis (usually  $\frac{1}{2}$  or  $\frac{1}{4}$  is ample). Return the remainder to the container as a reserve sample. Count, weigh, and powder the analyst's subdivision as directed under (a).

(c) *Containers of 100–500 units*.—If more than one container is available, count, weigh, and powder the entire contents of one of them. If only one container is available, but there is sufficient material to warrant subdividing, proceed as directed under (b); otherwise count, weigh, and powder the entire contents.

(d) *Small containers, such as tubes of hypodermic tablets*.—Choose a number of containers that will constitute a satisfactory sample; count, weigh, and powder the contents.

(e) *Tablets or pills of small dosages, for example, 1/100 grain of active ingredient*.—The number of units necessary may be so large as to render powdering unnecessary. An entire bottleful or half a bottleful may be required. Count the units to be employed and use them without powdering.

#### *II. Soft Capsules*

Count and weigh a representative number of capsules and ascertain the gross weight per capsule. Open the capsules and transfer as much of the contents as possible to a weighing bottle. Clean the capsules by cutting in two if necessary and washing by agitating with alternate portions of alcohol and ether. (A few drops of glacial acetic acid mixed with the alcohol aids in the cleaning.) Repeat until clean, finally removing the ether before a fan or air blast. Deduct the weight of the cleaned, empty capsules from the gross weight and calculate the average net contents.

#### *III. Ampuls*

Before opening dislodge any liquid adhering in neck. Mark with a file or other suitable instrument the level of the liquid on the necks of the requisite number of ampuls, open them near the tip, transfer the bulk of the contents to a small flask, and mix. To determine the volume of contents, wash and dry the empty ampuls and fill them to the mark with  $H_2O$  from a graduated pipet or buret.

#### ACETANILID AND ACETPHENETIDIN: (PHENACETIN)

2

#### *Qualitative Test for Acetphenetidin—Tentative*

To 0.001–0.002 g of the sample in a test tube add a drop of glacial acetic acid, 0.5 cc of  $H_2O$ , and 1 cc of 0.1 N I soln; warm the mixture to about  $40^\circ$  and add a drop of HCl. If acetphenetidin alone is present, its periodide separates almost immediately in the form of reddish brown leaflets or needle-like crystals. If the sample consists largely of acetanilid, the separation takes place on cooling and shaking the liquid. In the presence of considerable acetanilid, the periodide first separates as minute,

oily globules, which on vigorous shaking gradually become crystalline. By this test as little as 0.0005 g of acetphenetidin, if alone, may be detected in the form of its characteristic periodide.

*Quantitative Methods—Tentative*

## 3

## REAGENTS

(a) *Purified iodine*.—Dissolve 2 parts of resublimed I and 1 part of KI in 1 part of H<sub>2</sub>O, pour the clear soln into a large volume of H<sub>2</sub>O, filter, and wash the finely precipitated I several times on a perforated plate with H<sub>2</sub>O. Dry in the air and finally in a desiccator containing H<sub>2</sub>SO<sub>4</sub>. Store in a glass-stoppered weighing bottle.

(b) *Standard sodium thiosulfate soln*.—Dissolve 30 g of Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>·5H<sub>2</sub>O in recently boiled, cooled H<sub>2</sub>O and dilute to about 1 liter. Standardize this soln against the purified I as follows: Weigh accurately about 0.3 g of the purified I in a small glass capsule provided with a closely fitting glass cap or stopper. Place the capsule in a 200 cc Erlenmeyer flask containing 0.5 g of KI dissolved in 1–2 cc of H<sub>2</sub>O. After complete soln dilute with 10 cc of H<sub>2</sub>O and titrate with the Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> soln, using 1 or 2 drops of starch indicator, VI, 3(e).

(c) *Standard iodine soln*.—Dissolve 40 g of KI in the least possible quantity of H<sub>2</sub>O, add 30 g of I, and after solution dilute to about 1 liter. Standardize against the standard Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> soln.

## 4

## DETERMINATION

(a) *Acetphenetidin*.—(1) Place 0.2 g of the acetanilid-acetphenetidin mixture in a 50 cc lipped Erlenmeyer flask, add 2 cc of glacial acetic acid, heat gently over a wire gauze to complete soln, and dilute with 40 cc of H<sub>2</sub>O previously warmed to 70°. Transfer the clear liquid with two 10 cc portions of warm (40°) H<sub>2</sub>O to a glass-stoppered, 100 cc volumetric flask containing 25 cc of the standard I soln warmed to 40°. Stopper, mix thoroly by rotating the liquid, add 3 cc of HCl, continue rotating the liquid until crystallization begins, and then set aside to cool. (If the ratio of acetphenetidin to acetanilid is equal to or greater than unity, crystalline scales will form almost immediately on the addition of acid. As the proportion of acetanilid increases, however, the periodide tends to remain in the liquid state. Gentle agitation or rotation of the flask in H<sub>2</sub>O, warmed not to exceed 40°, hastens the formation of crystals.) When the contents are at room temp., fill the flask with H<sub>2</sub>O to within 2 or 3 cc of the mark, mix thoroly by rotating the mixture, and allow to stand overnight. Fill to the mark with H<sub>2</sub>O, mix thoroly, allow to stand 30 min., and filter thru a 5.5 cm dry, closely fitted filter into a 50 cc volumetric flask, rejecting about 15 cc of the first runnings but reserving it for the recovery of acetanilid. Transfer the 50 cc aliquot to a 200 cc Erlenmeyer flask and titrate the excess I with the standard Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> soln. Calculate the quantity of acetphenetidin from the following formula:

Acetphenetidin =  $I (0.0896 \times N)$ , in which

0.0896 = the number of g of acetphenetidin contained in 1 cc of a normal soln of this substance;

$N$  = the normality of the standard Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> soln used; and

$I$  = the number of cc of the standard Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> soln corresponding to the I combined with the acetphenetidin.

The formula of the precipitated periodide is (C<sub>7</sub>H<sub>7</sub>O·C<sub>6</sub>H<sub>4</sub>NH·COCH<sub>3</sub>)<sub>2</sub>HI·I.

(2) Determine acetphenetidin gravimetrically as follows: Filter off the periodide,

preferably by suction; wash with 10–15 cc of the standard I soln; and transfer the precipitate, together with the filter and any particles of the precipitate remaining in the volumetric flask, to a separator, using not over 50 cc of  $H_2O$ . Remove both free and added I with a few small crystals of  $Na_2SO_4$  and extract the liquid with three 50 cc portions of  $CHCl_3$ , washing each portion subsequently in a second separator with 5 cc of  $H_2O$ . After washing and clearing, filter the  $CHCl_3$  soln thru a dry 5.5 cm filter into a 200 cc Erlenmeyer flask, distil most of the  $CHCl_3$ , transfer the residual soln (5–10 cc) by means of a little  $CHCl_3$  to a small weighed beaker, evaporate to dryness on a steam bath, cool, and weigh.

(b) *Acetanilid*.—If the combined weight of the acetanilid-acetphenetidin mixture is known, determine the weight of the acetanilid by difference; or determine it directly from a second aliquot of the filtrate from the acetphenetidin periodide (a) as follows:

Pipet 25–30 cc of the clear liquid into a separator, decolorize with solid  $Na_2SO_3$ , and add solid  $NaHCO_3$  in slight excess, then 1 or 2 drops of acetic anhydride. Extract with three 60 cc portions of  $CHCl_3$ , passing the  $CHCl_3$  soln, when cleared, thru a small, dry filter into a 200 cc Erlenmeyer flask, and distil the  $CHCl_3$  by the aid of gentle heat to about 20 cc. Add 10 cc of  $H_2SO_4$  (1+9) and digest on a steam bath until the residue has been reduced one-half. Add 20 cc of  $H_2O$  and continue the digestion for an hour. Add a second 20 cc portion of  $H_2O$  and 10 cc of  $HCl$  and titrate very slowly, dropwise, with the standard bromide-bromate soln, 5(a), until a faint yellow color remains. While adding this reagent, rotate the flask sufficiently to agglomerate the precipitated tribromoanilin. Calculate the quantity of acetanilid present.

If the preparation contains caffeine or antipyrin, or both, in addition to acetanilid and acetphenetidin, proceed as follows: (1) Digest the mixture by heating with the  $H_2SO_4$  (1+9) to convert acetphenetidin and acetanilid to phenetidin and anilin sulfates, respectively, 7(a); (2) separate the caffeine and antipyrin by extraction with  $CHCl_3$ ; (3) regenerate acetphenetidin and acetanilid by treating the soln of the corresponding sulfates with solid  $NaHCO_3$  in slight excess and a few drops of acetic anhydride, and extract with  $CHCl_3$ .

#### ACETANILID AND CAFFEINE<sup>1</sup>—OFFICIAL, FIRST ACTION

##### 5

##### REAGENTS

(a) *Standard bromide-bromate soln*.—Dissolve 14 g of  $KBrO_3$  and 55 g of  $KBr$  in  $H_2O$ . Dilute to 1 liter and standardize against recrystallized and dried acetanilid, by one of the following procedures: (1) Proceed as directed under 4(b), beginning with "Add 10 cc of  $H_2SO_4$  (1+9)"; (2) transfer 10 cc of the soln to a glass-stoppered flask and add 25 cc of  $H_2O$ , 5 cc of 16.5%  $KI$  soln, and 5 cc of  $HCl$ . Shake thoroly and titrate the liberated I with 0.1  $N$   $Na_2S_2O_3$  soln, using starch soln as indicator, VI, 3(e).

(b) *Wagner's reagent*.—Dissolve 2 g of I and 6 g of  $KI$  in  $H_2O$  and dilute to 100 cc.

Treat all corks used in the distillation with  $CHCl_3$ .

##### 6

##### PREPARATION OF SOLUTION

(a) If the sample is already in powder, rub thoroly in a mortar and keep in a tightly corked tube or flask. Powders in paper, cachet, or capsule containers are frequently of such fineness as to require little further trituration except to produce a

uniform product. With tablets and pills, determine their average weight and powder in a mortar. Weigh 0.3–0.5 g of the sample or, if preferred, a quantity equal to, or a multiple of, the average unit dose (previously ascertained by weighing collectively 20 or more such doses). Transfer to a separator, add 50 cc of  $\text{CHCl}_3$  and 20 cc of  $\text{H}_2\text{O}$ , shake vigorously, and after clearing draw off the lower layer thru a small, dry filter into a 200 cc Erlenmeyer flask. Repeat the extraction twice, using 50 cc portions of  $\text{CHCl}_3$  for each extraction. Recover any caffeine-acetanilid mixture observable about the apex of the delivery tube of the separator, edge of filter, and tip of separator by careful washing with  $\text{CHCl}_3$  and add these washings to the main portion. Distil the combined  $\text{CHCl}_3$  extracts to about 10 cc.

If caffeine is present, as the free alkaloid or in other readily extractable form, the extraction may, if preferred, be made on filter paper by washing with successive 5–10 cc portions of  $\text{CHCl}_3$  (30–50 cc is usually sufficient) until the extraction is complete, as indicated by the absence of any residue after evaporation of a small portion of the last washing.

(b) With dilute alcoholic solns, evaporate a measured quantity on a steam bath until most of the alcohol has been expelled, or take an aliquot of the residue from an alcohol determination and transfer to a separator by pouring and rinsing with a minimum quantity of  $\text{H}_2\text{O}$  so that the final volume does not greatly exceed 20 cc. In order to avoid any loss of acetanilid by hydrolysis during evaporation, add a little solid  $\text{NaHCO}_3$  and a drop of acetic anhydride. Should the preparation contain other alkaloids, acidify with a few drops of  $\text{H}_2\text{SO}_4$  (1+9) immediately after acetylation to retain such basic material in the aqueous soln. Add 50 cc of  $\text{CHCl}_3$ , shake vigorously, and after clearing draw off the  $\text{CHCl}_3$  layer thru a filter into a 200 cc Erlenmeyer flask. Repeat the extraction twice, using 50 cc portions of  $\text{CHCl}_3$  for each extraction, and distil the combined  $\text{CHCl}_3$  washings to a volume of about 10 cc.

## 7

## DETERMINATION

(a) *Caffeine*.—Treat the  $\text{CHCl}_3$  soln obtained, 6, with 10 cc of  $\text{H}_2\text{SO}_4$  (1+9) and digest on a steam bath until the contents of the flask are reduced to 5 cc. Add 10 cc of  $\text{H}_2\text{O}$  and continue the digestion until the liquid is again reduced to 5 cc. (The diluting and evaporating process must be repeated until the odor of acetic acid can no longer be detected in the vapors.) Cool and transfer to a separator with a minimum of  $\text{H}_2\text{O}$ . (The final volume should not greatly exceed 20 cc.) Add 50 cc of  $\text{CHCl}_3$ , extract in the usual way, and after clearing withdraw the lower layer thru a small, dry filter into a 200 cc Erlenmeyer flask. Repeat the extraction with two 50 cc portions of  $\text{CHCl}_3$ . Distil the combined extracts to about 10 cc, finally transferring the residual liquid, by washing with  $\text{CHCl}_3$ , to a weighed beaker or crystallizing dish. Allow the soln to evaporate spontaneously, or by gentle heat and an air blast, to apparent dryness. Cool, and allow to stand in the open until weight becomes constant.

From those preparations that contain powdered cinnamon, celery seed, ginger, or other vegetable products,  $\text{CHCl}_3$  extracts, in addition to caffeine and acetanilid, certain oils, fats, waxes, resins, pigments, and other substances. After the caffeine-acetanilid mixture has been digested, these oils, etc., appear either in suspension or soln and contaminate the caffeine. Remove any suspended impurities by filtering thru a small, moistened filter immediately after hydrolysis and prior to extraction with  $\text{CHCl}_3$ . Should the recovered caffeine be deeply colored or contaminated with foreign matter, purify it as follows: Dissolve in  $\text{H}_2\text{SO}_4$  (about 5 cc of 0.2 *N* acid for

every 100 mg of caffeine); filter, if necessary, thru a moistened filter; and add 15–20 cc of Wagner's reagent, 5(b), sufficient to color distinctly the supernatant liquid a deep claret; stir, and allow to stand an hour, preferably in a refrigerator. Filter and wash the periodide with a few cc of I soln; transfer both filter and precipitate to a separator, using not more than 20 cc of  $H_2O$ ; and decolorize with a crystal of  $Na_2SO_3$ . Extract with three 50 cc portions of  $CHCl_3$  and proceed as directed above.

(b) *Acetanilid*.—Transfer the soln of anilin sulfate remaining in the separator to the Erlenmeyer flask used in effecting hydrolysis and heat 10 min. on a steam bath to expel all traces of  $CHCl_3$ . Wash the filter that was used in drying the  $CHCl_3$  soln of caffeine with 5 cc of  $H_2O$ , adding the washings to the main soln of anilin sulfate. Add 10 cc of  $HCl$  and titrate with the standard bromide-bromate soln, 5(a), until a faint yellow coloration remains, rotating the flask sufficiently to agglomerate the precipitated tribromoanilin. Calculate the acetanilid from the number of cc of the standard soln required.

(1) Add an excess of the standard bromide-bromate soln to the soln of anilin sulfate obtained under (b) and titrate the excess with 0.1  $N$   $Na_2S_2O_3$  after the addition of 5 cc of  $KI$  soln and starch soln as indicator, VI, 3(e). 1 cc of 0.1  $N$  bromide-bromate soln = 0.002252 g of acetanilid.

(2) To determine  $NaHCO_3$  also, which often appears as the  $CHCl_3$ -insoluble residue, titrate such residue with standard acid, using methyl orange indicator. The bicarbonate may also be determined by igniting the original sample (if talc is absent) or the  $CHCl_3$ -insoluble residue, with  $H_2SO_4$  and weighing the resulting  $Na_2SO_4$ .

(3) Should the "acetanilid compound" be combined with  $NaBr$ , the bromide, in the absence of other halides, may be determined volumetrically as directed under XII, 37. 1 cc of 0.1  $N$   $AgNO_3$  = 0.01029 g of  $NaBr$ .

#### ACETANILID, CAFFEINE, AND CODEINE—OFFICIAL, FIRST ACTION

##### 8

##### PREPARATION OF SOLUTION

Transfer to a separator one or more average unit doses (about 0.225 g of acetanilid) of the powdered sample; add 20 cc of  $H_2O$ , 50 cc of  $CHCl_3$ , and 10 drops of  $H_2SO_4$  (1+9); and extract in the usual way. After clearing, wash the solvent in a second separator with 5 cc of  $H_2O$  and transfer to a 200 cc Erlenmeyer flask. Repeat the foregoing operations with two 50 cc portions of  $CHCl_3$ , finally distilling the combined  $CHCl_3$  soln by gentle heat to about 10 cc.

##### 9

##### DETERMINATION

(a) *Acetanilid and caffeine*.—Treat the  $CHCl_3$  residue, 8, as directed under 7.

(b) *Codeine*.—Combine the wash  $H_2O$  used in the second separator under 8 with the soln of codeine sulfate. Add an excess of solid  $NaHCO_3$ , extract with 5 successive portions of 30, 25, 20, 15, and 10 cc of  $CHCl_3$ , wash the combined  $CHCl_3$  extracts with 5 cc of  $H_2O$  in a second separator, and pass thru a dry filter into a 200 cc Erlenmeyer flask. Distil by gentle heat to about 5 cc. Transfer the  $CHCl_3$  soln to a small weighed beaker, evaporate to apparent dryness on a steam bath, add a few drops of alcohol and a like quantity of  $H_2O$  to the amorphous residue, and evaporate again. Finally cool and allow the usually crystalline product to stand until the weight becomes constant. Check this result volumetrically by dissolving the residue in 3–5 cc of neutral alcohol and titrating with 0.02  $N$   $H_2SO_4$  to a faint red color, using methyl red indicator, II, 55(a).

The quantity of codeine found by weight will usually be slightly greater than that determined by titration. To insure the greatest possible accuracy in the volumetric operations, check the strength of the standard acid used by titration against pure codeine.

#### ACETANILID, CAFFEINE, AND QUININE—OFFICIAL, FIRST ACTION

##### 10

##### PREPARATION OF SOLUTION

Proceed as directed under 8.

##### 11

##### DETERMINATION

(a) *Acetanilid and caffeine*.—Proceed as directed under 7.

(b) *Quinine*.—Combine the wash  $H_2O$  used in the second separator under 8 with the soln of quinine bisulfate, add a slight excess of  $NH_4OH$ , and extract with three 50 cc portions of  $CHCl_3$ . Wash each portion with 5 cc of  $H_2O$  in a second separator and pass thru a dry filter into a 200 cc Erlenmeyer flask. Distil by gentle heat to about 5 cc, evaporate on a steam bath to apparent dryness, dissolve the amorphous alkaloid in 5 cc of neutral alcohol, and titrate with 0.02 *N* acid to a yellow color, using 2 drops of bromocresol purple indicator, 93. Heat on a steam bath until most of the alcohol has been expelled, adding, if necessary, sufficient acid to maintain the acid reaction. From the total number of cc of acid used in the titration calculate the quinine present. 1 cc of 0.02 *N* acid = 0.007565 g of quinine ( $C_{20}H_{24}O_2N_2 \cdot 3H_2O$ ) or 0.008726 g of quinine sulfate,  $(C_{20}H_{24}O_2N_2)_2 H_2SO_4 \cdot 7H_2O$ , or 0.007825 g of quinine sulfate,  $(C_{20}H_{24}O_2N_2)_2 H_2SO_4 \cdot 2H_2O$ , or 0.007934 g of quinine hydrochloride ( $C_{20}H_{24}O_2N_2HCl \cdot 2H_2O$ ) or 0.007943 g of quinine dihydrochloride ( $C_{20}H_{24}O_2N_2 \cdot 2HCl$ ).

If the mixture contains acetphenetidin in place of acetanilid, proceed as outlined above, except to conduct the separation of caffeine and acetphenetidin as directed under 17.

#### ACETANILID, CAFFEINE, QUININE, AND MORPHINE<sup>3</sup>—OFFICIAL, FIRST ACTION

##### 12

##### PREPARATION OF SOLUTION

Transfer to a separator a quantity (containing not less than 0.016 g of morphine) of the powdered sample and add 20 cc of  $H_2O$  and 10 drops of  $H_2SO_4$  (1+9). Extract with three 50 cc portions of alcohol-free  $CHCl_3$ , wash each portion in a second separator with 5 cc of  $H_2O$ , and add the combined washings to the alkaloidal soln in the first separator. Filter the  $CHCl_3$  extracts thru a small, dry filter into a 200 cc Erlenmeyer flask and distil by gentle heat to about 10 cc.

##### 13

##### DETERMINATION

(a) *Acetanilid and caffeine*.—Proceed as directed under 7, using the  $CHCl_3$  extract obtained under 12.

(b) *Quinine*.—Add to the soln of quinine and morphine sulfates, obtained under 12, 4–5 cc of a 10%  $NaOH$  soln and extract with four 40 cc portions of  $CHCl_3$ . Wash each portion with 5 cc of  $H_2O$  and pass the clear solvent thru a small, dry filter into a 200 cc Erlenmeyer flask. Remove the solvent by gentle distillation and titrate the residual quinine with 0.02 *N* acid as directed under 11(b). (If the morphine salt present is contaminated with codeine, the latter will be separated and titrated with the quinine.)

(c) *Morphine*.—Wash the filter used under (b) with 5 cc of  $H_2O$  and add the washings to the aqueous, alkaline soln of the alkaloid. Add 0.5 g of  $NH_4Cl$  (or a quantity slightly in excess of that required to free the morphine as well as convert all  $NaOH$  to  $NaCl$ ), 45 cc of  $CHCl_3$ , and 5 cc of alcohol. Extract in the usual way, washing the solvent in a second separator with 5 cc of  $H_2O$ . After clearing, pass the  $CHCl_3$  thru a small, dry filter into a 200 cc Erlenmeyer flask. Repeat the extraction with three 40 cc portions of  $CHCl_3$ , washing and filtering as before. Collect all the solvent in an Erlenmeyer flask and distil to about 10 cc. Transfer with  $CHCl_3$  to a small beaker and evaporate to apparent dryness. Dissolve the residue in 1–2 cc of warm, neutral methyl alcohol; add a drop of methyl red indicator, II, 55(a), and titrate with 0.02  $N$  acid to a faint red color. Evaporate most of the alcohol on a steam bath and, if necessary, add from a buret sufficient of the 0.02  $N$  acid to maintain the faint red color. From the total volume of 0.02  $N$  acid used calculate the morphine present. To insure the greatest possible accuracy, check the strength of the standard acid used by titration against pure morphine.

In the various operations involving fixation and subsequent liberation of morphine by means of fixed alkali and  $NH_4Cl$ , the most careful attention should be paid to the manner of adding the reagents, since any undue excess of either might nullify the entire procedure. Any large excess of  $NaOH$  would naturally require for its reduction a correspondingly large quantity of  $NH_4Cl$ , the latter in turn yielding its equivalent of hydroxide, relatively large quantities of which, thru interaction with  $NaCl$ , tend to inhibit any permanent liberation of alkaloid and thus prevent complete extraction. Furthermore,  $NH_4Cl$  in large quantity operates retentively on the morphine in soln, due in part possibly to the formation of an alkaloidal hydrochloride.

#### ACETANILID AND SODIUM SALICYLATE—OFFICIAL, FIRST ACTION

##### 14

##### PREPARATION OF SOLUTION

Weigh a quantity of the powdered sample equal to, or a multiple of, an average unit dose (about 0.225 g of acetanilid); transfer to a separator containing 10 cc of  $H_2O$ ; and for every unit dose add 0.1 g of solid  $NaHCO_3$ . In the case of tablets and pills, ascertain their average weight, powder in a mortar, and weigh a quantity of the powder equivalent to one or more tablets or pills for each determination. In the examination of alcoholic preparations, distil the alcohol from a measured volume on a steam bath, transfer to a separator with a minimum quantity of  $H_2O$ , and add 0.5–1.0 g of solid  $NaHCO_3$ .

##### 15

##### DETERMINATION

(a) *Acetanilid*.—Extract the alkaline soln obtained under 14 with three 50 cc portions of  $CHCl_3$ ; wash each portion with 5 cc of  $H_2O$  in a second separator and collect the solvent, without previous drying, in a 200 cc Erlenmeyer flask. Reserve the aqueous soln for the determination of  $Na$  salicylate, (b). Distil the  $CHCl_3$  very gently to about 5 cc, add 10 cc of  $H_2SO_4$  (1+9), and completely hydrolyze on a steam bath. Proceed from this point as directed under 7(b), beginning "Add 10 cc of  $HCl$ ."

(b) *Sodium salicylate*.—Acidify the aqueous soln of  $Na$  salicylate, (a), with a few drops of  $HCl$  and extract 3 to 5 times with 25 cc portions of  $CHCl_3$  to exhaust the salicylic acid present in the mixture. Treat each portion in a second separator with 20 cc of  $H_2O$  containing 1 g of anhydrous  $Na_2CO_3$  for every 0.1 g of salicylic acid. Shake vigorously, and after clearing wash each portion again in a third separa-



tor with 5 cc of  $H_2O$ . Add the washings to the main aqueous alkaline soln of Na salicylate. Dilute to a known volume; transfer an aliquot, representing about 0.1 g of salicylic acid, to a 200 cc Erlenmeyer flask; dilute to 60–75 cc; heat nearly to boiling; add slowly 50–80 cc of approximately 0.1  $N$  I soln, sufficient to insure an excess during digestion; and digest for an hour on a steam bath. Remove the free I with a few drops of  $Na_2S_2O_3$  soln and decant the clear liquid thru a weighed Gooch crucible, retaining most of the precipitate, tetraiodophenylenequinone  $(C_6H_2I_4O)_2$  in the flask. To the latter add 50 cc of boiling  $H_2O$ , digest 10 min. on a steam bath, filter, and gradually wash all the precipitate into the Gooch crucible, using for this purpose and the final washing about 200 cc of hot  $H_2O$ . Dry to constant weight in an air bath at  $100^\circ$ . Multiply the weight of the precipitate by 0.4654 to obtain the quantity of Na salicylate present in the aliquot taken.

Should the mixture contain caffeine or antipyrin, or both, these substances will appear with the acetanilid in the first  $CHCl_3$  extract and may be determined as directed in the remarks following 33(b). Should the acetanilid be replaced by acetphenetidin in the mixture, the general procedure would not be materially altered, the acetphenetidin being weighed directly after recovery from its washed  $CHCl_3$  soln as separated from the Na salicylate. If, instead of Na salicylate, the mixture contains the free acid or its  $NH_4$  salt, add a larger quantity of  $NaHCO_3$  prior to extraction with  $CHCl_3$  to insure the fixation of salicylic acid.

In the analysis of a mixture of caffeine, acetanilid, Na salicylate, and codeine, the following procedure is recommended: (1) Extraction of caffeine, acetanilid, and salicylic acid from the acidified soln; (2) washing the  $CHCl_3$  soln with aqueous  $Na_2CO_3$  soln for the recovery of the salicylic acid, preliminary to its treatment with I soln; (3) separation of caffeine and acetanilid as directed under 7(a) and 7(b); and (4) recovery of codeine from the soln of its sulfate after treatment with  $NaHCO_3$  and  $CHCl_3$ .

#### ACETPHENETIDIN (PHENACETIN) AND CAFFEINE<sup>1</sup>—TENTATIVE

##### 16

##### PREPARATION OF SOLUTION

In preparations containing acetphenetidin instead of acetanilid, but otherwise identical, make the gross separation of the caffeine-acetphenetidin mixture as directed under 6.

##### 17

##### DETERMINATION

(a) *Caffeine*.—Treat the  $CHCl_3$  extract obtained under 16 with 10 cc of  $H_2SO_4$  (1+9) and digest on a steam bath until the liquid is reduced to about 5 cc. Dilute with 10 cc of  $H_2O$  and continue the digestion until the volume is again reduced to 5 cc; again add 10 cc of  $H_2O$  and continue heating until the residual liquid amounts to 8–10 cc. The diluting and evaporating process must be repeated until the odor of acetic acid can no longer be detected in the vapors. If, during the digestion, particles of acetphenetidin remain on the sides of the flask, rinse them into the soln with a few drops of  $CHCl_3$ . (Great care must also be given to the degree of evaporation. Should the aqueous-acid soln and suspension of caffeine-acetphenetidin be concentrated much beyond the limits indicated, more or less phenetidin sulfonate is likely to be formed, which later resists acetylation and conversion to acetphenetidin.) Cool, transfer with  $H_2O$  to a separator so that the final volume does not greatly exceed 20 cc, and proceed as directed under 7(a).

(b) *Acetphenetidin*.—Wash the filter used to dry the  $CHCl_3$  with 5 cc of  $H_2O$ , receiving the washings in the separator containing the soln of phenetidin sulfate. Treat

with successive small portions of solid  $\text{NaHCO}_3$  until, after complete neutralization of free acid, an excess of  $\text{NaHCO}_3$  remains. Add 50 cc of  $\text{CHCl}_3$ , and for every 0.1 g of acetphenetidin known or believed to have been present, 5 drops of acetic anhydride. Shake vigorously, allow to clear, and withdraw the  $\text{CHCl}_3$  into a second separator containing 5 cc of  $\text{H}_2\text{O}$ . Shake this mixture, and after clearing pass the solvent thru a small, dry filter into a 250 cc Erlenmeyer flask. Repeat the extraction twice with 50 cc portions of  $\text{CHCl}_3$ , washing each portion with the 5 cc of  $\text{H}_2\text{O}$  in the second separator. Distil the combined  $\text{CHCl}_3$  extractions to about 10 cc, transfer the residual soln with sufficient fresh solvent to a weighed 50 cc beaker or crystallizing dish, evaporate on a steam bath to apparent dryness, and finally remove any considerable excess of acetic anhydride by repeated additions and evaporations of 1 cc of  $\text{CHCl}_3$  and a drop of alcohol. (The reformed acetphenetidin should finally appear as a whitish, crystalline mass with a faint, acetous odor that disappears completely on standing some hours in the open or over lime in a vacuum desiccator.) Weigh at intervals until the final weight differs from the preceding by not more than 0.0005 g.

#### ACETPHENETIDIN (PHENACETIN) AND SALOL<sup>1</sup>

18

##### *Acid Hydrolysis Method—Tentative*

(a) *Acetphenetidin*.—Weigh on a tared 5.5 cm filter a quantity of the sample equal to, or a multiple of, the average weight of a unit dose and wash with sufficient successive small portions of  $\text{CHCl}_3$  to extract completely all acetphenetidin and salol present in the mixture (about 40 cc). Collect the soln in a weighed, 100 cc beaker and evaporate on a warm plate ( $50-60^\circ$ ) to apparent dryness, using an air blast. Let stand 24 hours at room temp. to practically constant weight and weigh. By means of  $\text{CHCl}_3$  transfer the crystalline residue to a 50 cc lipped Erlenmeyer flask, evaporate the solvent by means of an air blast and gentle heat, add 10 cc of  $\text{H}_2\text{SO}_4$  (1+9) and evaporate on a steam bath until the volume is reduced one-half. Add 10 cc of  $\text{H}_2\text{O}$  and continue the digestion as before. Add a second 10 cc of  $\text{H}_2\text{O}$  and evaporate to 5 cc. Transfer the residue with about 20 cc of  $\text{H}_2\text{O}$  to a small separator and extract the salol with 15, 10, and 5 cc of  $\text{CHCl}_3$ , washing each extract with 5 cc of  $\text{H}_2\text{O}$  in a second separator to recover traces of phenetidin sulfate possibly dissolved by the  $\text{CHCl}_3$ . Add the wash water in the second separator to the solution of phenetidin sulfate in the first separator and proceed as directed under 17(b), beginning with "Treat with successive small portions of solid  $\text{NaHCO}_3$ ."

(b) *Salol*.—Subtract the weight of acetphenetidin from the combined weight of the two ingredients to obtain the weight of salol.

19

##### *Alkaline Hydrolysis Method—Tentative*

(a) *Acetphenetidin*.—On a small, tared filter or in a small beaker weigh a quantity of the sample containing not more than 0.08 g of salol; exhaust with  $\text{CHCl}_3$  as directed under 18(a); collect the solvent in a small, lipped Erlenmeyer flask; and evaporate the  $\text{CHCl}_3$  by means of an air blast without heat. Add 10 cc of 2.5%  $\text{NaOH}$  soln and heat 5 min. on a steam bath. Cool quickly to room temp. in running  $\text{H}_2\text{O}$  to prevent partial hydrolysis of the acetphenetidin. Transfer the liquid to a separator with a minimum quantity of  $\text{H}_2\text{O}$  and rinse the flask with the first 20 cc portion of  $\text{CHCl}_3$  to be used in the following extraction. Extract the alkaline soln with three 20 cc portions of  $\text{CHCl}_3$ ; wash each portion in a second separator with 5 cc of  $\text{H}_2\text{O}$ , and pass the  $\text{CHCl}_3$  soln thru a small, dry filter into a 200 cc Erlenmeyer

flask. Reserve the combined alkaline soln and washings for the determination of salol (b). Distil the combined  $\text{CHCl}_3$  extracts to about 5 cc. Transfer by means of a little  $\text{CHCl}_3$  to a small, weighed beaker or crystallizing dish, evaporate on a steam bath with the aid of an air blast, cool, and weigh the residual acetphenetid in at intervals until the weight becomes constant.

(b) *Salol*.—Place the reserved combined alkaline soln and washings under (a) in a 500 cc glass-stoppered bottle, dilute with  $\text{H}_2\text{O}$  to about 200 cc, run in from a buret an excess (about 50 cc) of 0.1 *N* bromide-bromate soln, add 10 cc of  $\text{HCl}$ , and shake 1 min., then at intervals for 30 min. Add 10 cc of 15%  $\text{KI}$  soln and shake at intervals for 15 min. Titrate the free  $\text{I}$  with standard  $\text{Na}_2\text{S}_2\text{O}_3$  soln previously standardized against the 0.1 *N* bromide-bromate soln, 26(c). 1 cc of 0.1 *N* bromide-bromate soln = 0.001784 g of salol.

#### ACETYSALICYLIC ACID<sup>a</sup>

20

##### *Melting Point—Official*

If excipients are present, treat 0.2–0.3 g with small portions of  $\text{CHCl}_3$  and filter into a beaker or evaporating dish. Evaporate the bulk of the  $\text{CHCl}_3$  on a steam bath and complete by spontaneous evaporation until thoroly dry. To determine the melting point of the crystalline residue use the U.S.P. method.

#### FREE SALICYLIC ACID

21

##### *Qualitative Test—Official*

Shake a 0.5 g sample in a small Erlenmeyer flask with about 10 cc of  $\text{CHCl}_3$  and filter. Evaporate, treat the residue with 10 cc of cold  $\text{H}_2\text{O}$ , and filter. Add 1 drop of a 10%  $\text{FeCl}_3$  soln. Only a very faint violet color should result.

##### *Quantitative Method—Official*

22

#### REAGENTS

(a) *Standard salicylic acid soln*.—Dissolve 0.01 g of salicylic acid in 100 cc of alcohol. Use only a freshly prepared soln.

(b) *Ferric ammonium sulfate*.—Add 1 cc of normal  $\text{HCl}$  to 2 cc of 8%  $\text{FeNH}_4(\text{SO}_4)_2 \cdot 12 \text{H}_2\text{O}$  soln and dilute with  $\text{H}_2\text{O}$  to 100 cc.

23

#### PREPARATION OF SAMPLE

Weigh 100 tablets for average weight, triturate in a mortar to a fine powder, and keep in a tightly stoppered bottle.

24

#### DETERMINATION

In each of two colorimeter tubes mix 48 cc of  $\text{H}_2\text{O}$  and 1 cc of the freshly prepared ferric ammonium sulfate. Shake 2.5 g of the powdered sample with exactly 25 cc of alcohol and filter if necessary. Immediately add 1 cc of the filtrate to one of the colorimeter tubes and 1 cc of the standard salicylic acid soln to the other, and mix. Immediately and rapidly make color comparisons and calculate the free salicylic acid on the basis of the acetylsalicylic acid present. If the color is too intense for satisfactory comparison, repeat the entire determination, using a smaller weight of the powdered sample.

## TOTAL SALICYLATES

25

*Iodine Method<sup>3</sup>—Official*

Weigh a 0.1 g sample into a 200 cc Erlenmeyer flask, add 20 cc of  $H_2O$  and 1 g of  $Na_2CO_3$ , and heat on a steam bath for 15 min. Filter, if necessary, to remove tale. Dilute to 60–75 cc, heat nearly to boiling, add slowly an excess (50–80 cc) of approximately 0.1 N I soln, and proceed as directed under 15(b) (sodium salicylate). Multiply the weight of the precipitate by 0.4015 to obtain the total salicylic acid and deduct the free salicylic acid as determined under 24. Multiply the remainder by 1.304 to obtain the weight of acetylsalicylic acid.

*Bromine Method—Official*

26

## REAGENTS

- (a) *Sodium hydroxide soln.*—Dissolve 2 g of NaOH in  $H_2O$  and dilute to 100 cc.
- (b) *Potassium iodide soln.*—Dissolve 20 g of KI in  $H_2O$  and dilute to 100 cc.
- (c) *Standard bromide-bromate soln (0.1 N bromine soln).*—Dissolve 3 g of  $KBrO_3$  and 12 g of KBr in  $H_2O$  and dilute to 1 liter. Standardize 30 cc of the Br soln by transferring to a glass-stoppered Erlenmeyer flask and adding 25 cc of  $H_2O$ , 5 cc of KI soln, and 5 cc of HCl. Shake thoroly. Titrate with 0.1 N  $Na_2S_2O_3$  soln, using starch indicator, VI, 3(e).

27

## DETERMINATION

Saponify a 0.5 g sample with 10 cc of the NaOH soln by heating for 15 min. on a steam bath. Dilute with  $H_2O$  in a volumetric flask to 500 cc. Transfer an aliquot of this soln, representing not less than 0.04 g nor more than 0.05 g of acetylsalicylic acid, to a 500 cc glass-stoppered Erlenmeyer flask, add 30 cc of the standard bromide-bromate soln and 5 cc of HCl, and immediately insert the stopper. Shake repeatedly for 30 min. and allow to stand for 15 min. Remove the stopper just sufficiently to introduce quickly 5 cc of the KI soln, taking care that no Br vapors escape, and immediately stopper the flask. Shake thoroly, remove the stopper, and rinse it and the neck of the flask with a little  $H_2O$  so that the washings flow into the flask. Titrate with 0.1 N  $Na_2S_2O_3$  soln, using starch indicator, VI, 3(e). 1 cc of 0.1 N bromide-bromate soln = 0.0023 g of salicylic acid, or 0.003 g of acetylsalicylic acid.

*Double Titration Method for Acetylsalicylic Acid<sup>3e</sup>—Official*

28

## PREPARATION OF SOLUTION

(a) *Dry extraction method (applicable in all cases).*—Treat a quantity of sample containing not less than 0.3 g of acetylsalicylic acid, accurately weighed, with small portions of  $CHCl_3$ , filter into a beaker, and wash the residue with  $CHCl_3$  until completely extracted. Evaporate the bulk of the  $CHCl_3$  on a steam bath, finishing with the aid of an electric fan without heat.

(b) *Wet extraction method (applicable in the absence of acids and alkalis, or alkaline earth carbonates).*—Transfer the accurately weighed sample to a small separator containing about 20 cc of  $H_2O$ . Shake out repeatedly with  $CHCl_3$ , using successively 30, 25, 20, 15, 10, and 5 cc portions, and test for completeness of extraction by evaporating on a watch-glass a portion of the final extraction. Filter the combined  $CHCl_3$  portions thru cotton and wash the funnel and cotton with  $CHCl_3$ . Evaporate the

bulk of the  $\text{CHCl}_3$  on a steam bath, finishing with the aid of an electric fan without heat.

(c) *Acetylsalicylic acid and uncoated tablets containing no excipient.*—Dissolve the sample directly in 10 cc of neutral alcohol.

## 29

## DETERMINATION

Dissolve the dry  $\text{CHCl}_3$  extract in 10 cc of neutral alcohol and titrate immediately and rapidly with 0.1 *N* alkali soln, using phenolphthalein indicator. Use the first persistent pink color as the end point, since any slight excess of alkali has a tendency to hydrolyze the ester quickly. Add a volume of the 0.1 *N* alkali equal to that used in the first titration and then add 5 cc more. Heat on a steam bath for 15 min. Titrate back with 0.1 *N* acid. If the product is pure, the total quantity of alkali consumed will be twice that of the first titration. Each cc of 0.1 *N* alkali consumed in the 2 titrations = 0.009 g of acetylsalicylic acid.

## 30

COMBINED ACETIC ACID IN ACETYSALICYLIC ACID<sup>11</sup>—OFFICIAL

Weigh accurately 2 g of the powdered material and transfer to a separator, using about 25 cc of  $\text{H}_2\text{O}$ . Extract completely with  $\text{CHCl}_3$ , testing the last extraction by evaporating a small quantity of the  $\text{CHCl}_3$  to dryness. (Usually 6 extractions with 30, 25, 20, 10, 10, and 5 cc portions of  $\text{CHCl}_3$  are sufficient.) Collect the  $\text{CHCl}_3$  fractions in a beaker and filter thru a pledget of cotton into a weighed beaker that has been counterpoised previously with a beaker of the same dimensions, similarly dried, and exposed to the air. Wash the original beaker, funnel, and cotton with  $\text{CHCl}_3$  and add these washings to the  $\text{CHCl}_3$  soln in the weighed beaker. Evaporate the  $\text{CHCl}_3$  on a steam bath, dry the residue at  $80^\circ$  for 15 min., and weigh, using the counterpoised beaker similarly treated. From the weight calculate the  $\text{CHCl}_3$  extract.

Treat the  $\text{CHCl}_3$  extract, or if no excipients are present, 2 g of the powdered material, in a 150 cc beaker with 30 cc of *N*  $\text{NaOH}$  and evaporate on a steam bath nearly to dryness. Transfer to a separator, using for this operation 10 cc of  $\text{H}_2\text{O}$ , 20 cc of 10%  $\text{H}_2\text{SO}_4$ , and finally two 5 cc portions of  $\text{H}_2\text{O}$ . Extract with successive portions of  $\text{CHCl}_3$ , using the first fraction of 50 cc to rinse the beaker in which the saponification was carried on. Continue the extractions with  $\text{CHCl}_3$  until all salicylic acid is removed (about 6 extractions). During these extractions keep the stopper in the separator to guard against loss of acetic acid by evaporation. Collect the  $\text{CHCl}_3$  fractions in a second separator, wash with 25 cc of  $\text{H}_2\text{O}$ , and wash this  $\text{H}_2\text{O}$  once with 5 cc of  $\text{CHCl}_3$ . Discard the  $\text{CHCl}_3$  extractions and return the wash  $\text{H}_2\text{O}$  to the acid  $\text{H}_2\text{O}$  in the first separator. Transfer the acid  $\text{H}_2\text{O}$  containing acetic acid and  $\text{H}_2\text{SO}_4$  to a 200 cc volumetric flask, wash the separators thoroly with  $\text{H}_2\text{O}$ , add to the flask, dilute to volume, and mix thoroly. Pipet two 50 cc portions, using the same pipet and draining it the same length of time. Place one portion in a receptacle suitable for titration and the other in a large Pt dish. Titrate the first portion at once with 0.5 *N* alkali, using phenolphthalein indicator. Evaporate the portion in the Pt dish on a steam bath to dryness, take up in 10 cc of  $\text{H}_2\text{O}$ , and again evaporate, repeating this process twice more. (During evaporation guard against contact with  $\text{NH}_3$  vapors.) Take up the residue in  $\text{H}_2\text{O}$  and titrate with 0.5 *N* alkali, using phenolphthalein indicator. Subtract the second titration reading from the first and calculate the percentage of acetic acid on a 0.5 g sample. 1 cc of 0.5 *N* alkali = 0.03002 g of acetic acid.

### 31 ACETYSALICYLIC ACID IN MIXTURES CONTAINING ACETPHENETIDIN AND CAFFEINE<sup>1</sup>—OFFICIAL

Ascertain the average weight of a number of tablets and reduce to a fine powder.

Weigh accurately 0.2 g of the powder, transfer to a separator with about 25 cc of  $H_2O$ , and extract carefully with repeated portions of  $CHCl_3$ . Test the final extraction by evaporating a small portion on a steam bath to dryness. (No residue should remain if the extraction is complete. About 6 extractions are generally required, and these can be made with 30, 25, 20, 10, 10, and 5 cc portions of  $CHCl_3$ .) Collect the  $CHCl_3$  fractions in a separator and draw off into a 200 cc Erlenmeyer flask, placing a pledget of cotton in the stem of the separator to filter the  $CHCl_3$ . Wash the separator twice with 5 cc portions of  $CHCl_3$ , passing this thru the cotton and leaving any  $H_2O$  that may have separated in the separator. Add the  $CHCl_3$  washings to the flask and evaporate the  $CHCl_3$  on a steam bath to a volume of about 2 cc. Add 10 cc of  $H_2SO_4$  (1+9), connect with a reflux condenser, and digest for 30 min., partially immersing the flask in a boiling water bath. Cool, and transfer to a separator, rinsing the condenser with  $CHCl_3$  and using a minimum quantity of  $H_2O$  to effect the transfer, so that the final volume does not greatly exceed 20 cc. Extract the caffeine and salicylic acid with 6 portions of  $CHCl_3$ , using 30, 25, 20, 15, 10, and 10 cc for the extractions. Collect these fractions in a separator, add 20 cc of  $H_2O$  and 1 g of  $Na_2CO_3$ , and shake thoroly. Drain off the  $CHCl_3$  into another separator and wash twice more with 15 and 10 cc of  $H_2O$ . Reject the  $CHCl_3$  and combine the  $Na_2CO_3$  soln and wash waters in a 200 cc Erlenmeyer flask. Heat on a steam bath to expel traces of  $CHCl_3$ , dilute to 100 cc with  $H_2O$ , then add slowly 25–40 cc of strong I soln (about 0.2 N), sufficient to insure excess during digestion, and digest 1 hour on the steam bath. Remove the free I with a few drops of  $Na_2S_2O_3$  soln. Decant the clear soln thru a weighed Gooch, retaining most of the precipitate in the flask. To the latter add 50 cc of boiling  $H_2O$ , digest 10 min. on the steam bath, filter, and wash gradually all the precipitate into the Gooch, using altogether about 200 cc of hot  $H_2O$  to complete the operation. Dry to constant weight in an air bath at  $100^\circ$  and weigh the precipitate of tetraiodophenylenequinone,  $(C_6H_2I_4O)_2$ . Weight of precipitate  $\times 0.4016$  = the total salicylic acid present. If free salicylic acid is present, deduct from the total; the difference  $\times 1.304$  = the weight of acetylsalicylic acid.

### ANTIPYRIN AND CAFFEINE<sup>2</sup>—OFFICIAL, FIRST ACTION

#### 32

#### PREPARATION OF SOLUTION

(a) Weigh a quantity of the finely powdered sample equal to, or a multiple of, an average unit dose; transfer to a filter and extract with  $CHCl_3$  to separate the caffeine and antipyrin from the usual excipients of tablet and pill combinations. Distil the greater part of the  $CHCl_3$  and evaporate the remainder on a steam bath.

(b) With alcoholic preparations, remove the alcohol from a measured quantity of the sample by heating on a steam bath. Extract the residue with three 50 cc portions of  $CHCl_3$  in a separator. Distil the greater portion of the  $CHCl_3$  and evaporate the remainder on a steam bath.

#### 33

#### DETERMINATION

(a) *Antipyrin*.—Transfer the residue obtained under 32, which should weigh about 0.25 g, to a 125 cc separator by means of two 5 cc portions of alcohol-free  $CHCl_3$ , followed by 10 cc of  $H_2O$ . Add 1 g of  $NaHCO_3$  and 10–15 cc of 0.2 N I (or double the quantity of 0.1 N I), adding the latter in small portions and shaking the

mixture vigorously after each addition. (The I should then be in excess of that required to convert all the antipyrin into the mono-iodo derivative. If it is not, add a little more I and shake the mixture again.) Remove the free I with a small crystal of  $\text{Na}_2\text{S}_2\text{O}_3$  and add 15 cc of washed  $\text{CHCl}_3$ , shaking vigorously for 1 min. After clearing, draw off the  $\text{CHCl}_3$  soln into a second separator; wash with 5 cc of  $\text{H}_2\text{O}$ , filter thru a small, dry filter into a weighed 50 cc beaker, and evaporate to apparent dryness on a steam bath, using an air blast. Repeat the extraction with two (3, if 0.1 N I has been used) 25 cc portions of washed  $\text{CHCl}_3$ , wash, filter, and evaporate each portion as above. Recover any crystalline product separating about the tip of the delivery tube, funnel, and edge of filter by judicious washing with  $\text{CHCl}_3$ . Dry the nearly colorless, crystalline residue of caffeine and iodoantipyrin 30 min. at  $100^\circ$ , cool, and weigh. Designate this weight as "A."

The use of alcohol-free  $\text{CHCl}_3$  in connection with the halogenation of antipyrin is necessary in order to preclude the formation of  $\text{CHI}_3$ , the presence of which in the composite residue A would vitiate the result.

Dissolve the composite residue in 5 cc of glacial acetic acid, add 10 cc of saturated  $\text{SO}_2$  soln, and transfer with hot  $\text{H}_2\text{O}$  to a 400–500 cc beaker until the final volume amounts to about 200 cc. Add sufficient  $\text{AgNO}_3$  soln to precipitate all the I (about 0.3 g of  $\text{AgNO}_3$ ) and a few drops of  $\text{HNO}_3$ , heat nearly to boiling, and stir to agglomerate the AgI. Add 15 cc of  $\text{HNO}_3$ , cover the beaker with a watch-glass, and boil gently for 5 min. Filter by decantation thru a weighed Gooch crucible; wash the precipitate once with a little alcohol, then with two 100 cc portions of boiling  $\text{H}_2\text{O}$ ; and finally transfer the silver iodide to the crucible. Wash several times with hot  $\text{H}_2\text{O}$  and again with alcohol to remove traces of organic matter, dry 30 min. in an air bath at  $110^\circ$ , cool, and weigh. Weight of AgI  $\times 0.8014$  = the weight of antipyrin.

(b) *Caffeine*.—Multiply the weight of AgI by 1.3374 and subtract the product from the weight A, under (a).

In the analysis of a mixture containing caffeine, antipyrin, acetanilid, and Na salicylate, the following steps are essential in effecting a separation: (1) Extraction of caffeine, acetanilid, and antipyrin from the aqueous, alkaline soln with  $\text{CHCl}_3$ ; (2) hydrolytic treatment with  $\text{H}_2\text{SO}_4$  of the three substances thus separated preliminary to the determination of caffeine and antipyrin as directed under a.

## 34

PILOCARPINE HYDROCHLORIDE<sup>a</sup>—OFFICIAL, FIRST ACTION

Ascertain the average weight per tablet. Pulverize, mix thoroly, and weigh out a sufficient portion to represent 1 grain of the salt. Dissolve the sample in 10 cc of  $\text{H}_2\text{O}$ , add 1 cc of 10%  $\text{NH}_4\text{OH}$ , and shake out rapidly with 20 cc of  $\text{CHCl}_3$ . Repeat the extraction, using 15 cc of  $\text{CHCl}_3$ , and complete with successive 10 cc portions. Filter each portion of the  $\text{CHCl}_3$  drawn off thru a pledget of cotton and combine in a 250 cc beaker, finally washing the stem of the separator and funnel with  $\text{CHCl}_3$ . Evaporate on the steam bath until the chloroform soln measures about 5 cc. Add 20 cc of 0.02 N  $\text{H}_2\text{SO}_4$  and evaporate the remainder of the  $\text{CHCl}_3$ . Titrate the excess acid with 0.02 N  $\text{NaOH}$ , using 1 drop of methyl red as indicator. (The end point is not particularly sharp, but with care it can be obtained.) 1 cc of 0.02 N  $\text{H}_2\text{SO}_4$  = 0.004893 g of  $\text{C}_{11}\text{H}_{15}\text{O}_2\text{N}_2 \cdot \text{HCl}$ .

EMETINE HYDROCHLORIDE IN TABLETS<sup>a</sup>—OFFICIAL, FIRST ACTION

## 35

## PREPARATION OF SAMPLE

Weigh collectively at least 50 unbroken tablets and calculate the average weight per tablet. Powder a representative number of tablets and mix thoroly.

36

## DETERMINATION

Transfer to a small separator sufficient of the powdered material, accurately weighed, to represent approximately 0.1 g of the alkaloidal salt. Dissolve in a minimum of  $H_2O$  and add 5 cc of 4% NaOH soln. Extract with 30 cc of washed ether, draw off the aqueous soln, and swirl the separator to remove the  $H_2O$  from the sides. Wash the ether with 1 cc of  $H_2O$ , adding the wash  $H_2O$  to the aqueous soln. Decant the ether into a third separator, washing the mouth of the separator with ether. Repeat the extractions with 25, 20, 15 and 10 cc portions of ether or until extraction is complete, washing with 1 cc of  $H_2O$  each time, and combine the ether extracts in the third separator. Filter into a beaker thru cotton previously wet with ether, finally wash the separator with ether, and evaporate on the steam bath, using a low temp. to complete the evaporation.

To the residue add 2 cc of neutral alcohol, cover the beaker with a watch-glass, and allow to reflux on the steam bath for a few min. Add a few drops of methyl red indicator, II, 55(a), and without dilution titrate with 0.02 *N* acid to a faint pink. Cover the beaker and digest on a steam bath until all particles are completely dissolved. Cool, and add about 30 cc of recently boiled distilled  $H_2O$ . Finish the titration with standard acid to a faint red. 1 cc of 0.02 *N*  $H_2SO_4 = 0.005533$  g of emetine hydrochloride ( $C_{20}H_{40}O_4N_2 \cdot 2HCl$ ).

37

ATROPINE IN TABLETS<sup>14</sup>—OFFICIAL, FIRST ACTION

Weigh 25-100 tablets and introduce directly into a small separator. Dissolve in 5-20 cc of  $H_2O$  and add 1 cc of  $NH_4OH$ . Add an equal volume of  $CHCl_3$ , agitate, and allow to stand until separation is complete. Draw off the  $CHCl_3$  layer into a second separator and repeat the extraction with fresh portions of the solvent until the alkaloid is completely removed. After combining all the fractions, wash the combined  $CHCl_3$  solns by agitation with 5 cc of  $H_2O$  and allow to stand 15 min. Introduce a pledget of absorbent cotton into the stem of the separator and carefully draw off the  $CHCl_3$  soln into a small beaker, but do not allow the wash  $H_2O$  to enter the orifice of the stopcock. Add 10 cc of  $CHCl_3$ , agitate, and when the  $H_2O$  has entirely risen to the surface draw off the  $CHCl_3$  into the beaker. Wash the outer surface of the stem of the separator with a little  $CHCl_3$ , adding the washings to the beaker. Evaporate the soln on the steam bath to about 5 cc. Add a measured excess volume of 0.02 *N*  $H_2SO_4$ , and continue the evaporation until the odor of  $CHCl_3$  has disappeared. Cool the soln and titrate back with 0.02 *N* NaOH, using 1 drop of methyl red indicator. 1 cc of 0.02 *N*  $H_2SO_4 = 0.005784$  g of atropine or 0.006945 g of atropine sulfate.

## CINCHONA ALKALOIDS

QUININE, CINCHONIDINE, CINCHONINE, AND QUINIDINE<sup>17</sup>—TENTATIVE

38

## REAGENTS

(a) *Acidified Rochelle salt soln.*—To each 100 cc of a saturated soln of Rochelle salt add 3 cc of 0.225 *N*  $H_2SO_4$ .

(b) *Washing soln.*—Dilute (a) with an equal volume of  $H_2O$ .

39

## DETERMINATION

Weigh a quantity of the sample sufficient to give approximately 0.5 g of total alkaloids, dissolve in 10%  $H_2SO_4$ , filter if necessary, add an excess of  $NH_4OH$ , and



extract with  $\text{CHCl}_3$  until the alkaloids are completely removed. Evaporate the combined  $\text{CHCl}_3$  extractions on a steam bath, add 5 cc of absolute alcohol and again evaporate, dry at  $110^\circ$ , and weigh.

Dissolve the residue in 50 cc of the 0.225  $N$   $\text{H}_2\text{SO}_4$ , heat on a steam bath for 10 min., and make just alkaline (shown by a faint permanent precipitate) with a 5% soln of  $\text{NaOH}$  added cautiously and with stirring. Add sufficient 0.225  $N$   $\text{H}_2\text{SO}_4$  to clear the soln and then add 5 cc in excess. Add 25 cc of the Rochelle salt soln and stir to start precipitation. Remove from the steam bath and place in an ice box at  $10$ – $15^\circ$ , stirring occasionally for 2 hours. Filter, and wash with 40 cc of the cold washing soln, using a small wash bottle and stirring the precipitate on the filter with a rubber-tipped glass rod to remove all soluble alkaloidal salts. Designate the combined filtrate and washings as soln "A" and save for determination of quinidine and cinchonine. Decompose the precipitate of quinine and cinchonidine tartrates with warm 10%  $\text{H}_2\text{SO}_4$  and transfer to a separator, washing the filter thoroly to remove all alkaloids. Make alkaline with  $\text{NH}_4\text{OH}$  and remove alkaloids completely by successive extractions with  $\text{CHCl}_3$ . (Four extractions with 25, 20, 15, and 10 cc portions, respectively, are usually sufficient.) Evaporate the combined  $\text{CHCl}_3$  extractions in a weighed beaker containing a little sharp sand, add 5 cc of absolute alcohol, evaporate to dryness on a steam bath, heat for 3 hours at  $100^\circ$ , cool, and weigh the mixed anhydrous alkaloids, quinine and cinchonidine. Add exactly 1 cc of the 0.225  $N$   $\text{H}_2\text{SO}_4$  for each 0.015 g of alkaloids and when completely dissolved transfer the soln to a polariscope tube, filtering if necessary. Use the longest tube possible, reducing its capacity when only small quantities of soln are available by inserting a straight tube of small bore slightly shorter than the polariscope tube and fastened securely as to center. Use a bichromate filter and correct the instrument for the operator's eyes. Great precision is necessary for accurate determinations. Read at  $20^\circ$  and calculate to a basis of a 100 mm tube. If the Ventzke scale is used, calculate to angular degrees by multiplying the reading by the factor 0.34657.

(a) *Quinine*.—Calculate the percentage of quinine in the total anhydrous alkaloids obtained in the tartrate separation from the following formula, which is based on the specific rotations of quinine ( $-277.4^\circ$ ) and cinchonidine ( $-180^\circ$ ) and the observed rotation of the mixed alkaloids at  $20^\circ$  calculated to a 100 mm tube.

$$Q = (-68.44) (a + 2.7), \text{ in which}$$

$Q$  = percentage of quinine in the total anhydrous mixed alkaloids obtained in the tartrate separation; and

$a$  = the observed angular rotation, calculated to a 100 mm basis.

(b) *Cinchonidine*.—Determine cinchonidine by difference.

Calculate these results to percentage basis of original sample.

(c) *Quinidine*.—Place soln A on a steam bath for 10 min., add 0.5 g of  $\text{KI}$ , remove from the steam bath, and place in the ice box at  $10$ – $15^\circ$  for 2 hours, stirring occasionally. Filter on a weighed Gooch crucible and wash with 15 cc of ice  $\text{H}_2\text{O}$ , saving the filtrate and washings (soln B). Dry the precipitate of neutral quinidine hydriodide at  $100^\circ$  for 1 hour (a slight yellowing does not affect the results), cool, and weigh. Weight of quinidine hydriodide  $\times 0.717$  = weight of anhydrous quinidine alkaloid.

(d) *Cinchonine*.—Transfer soln B to a separator, make alkaline with  $\text{NH}_4\text{OH}$  and extract completely with  $\text{CHCl}_3$ . Combine the  $\text{CHCl}_3$  extractions in a weighed beaker containing a trace of sharp sand, add 5 cc of absolute alcohol, evaporate to dryness, heat at  $100^\circ$  for 1 hour, cool, and weigh as anhydrous cinchonine alkaloid.

*Constants of Cinchona Alkaloids*

ALKALOID	SPECIFIC ROTATION IN ABSOLUTE ALCOHOL	APPROXIMATE SOLUBILITY IN ETHER	THALLEIOQUIN TEST*
Quinine	levo 168°	1 g in 19 cc	Positive
Quinidine	dextro 256°	1 g in 67 cc	Positive
Cinchonine	dextro 225°	1 g in 526 cc	Negative
Cinchonidine	levo 108°	1 g in 188 cc	Negative

\* *Thalleioquin Test*: Add 1 or 2 drops of Br water to 5 cc of an aqueous soln of the alkaloidal salt (1 in 1000) and 1 cc of  $\text{NH}_4\text{OH}$  (10%). The liquid acquires an emerald-green color due to the formation of thalleioquin.

## 41

EPHEDRA ASSAY<sup>13</sup>—OFFICIAL

Place 10 g of ephedra, in fine powder, in an Erlenmeyer flask. Add exactly 100 cc of solvent consisting of 3 volumes of ether and 1 volume of  $\text{CHCl}_3$  cooled to working temp. after mixing. Stopper securely, shake, and allow to stand at least 5 min. Add 5 cc of 10%  $\text{NH}_4\text{OH}$  and 0.5 g of anhydrous  $\text{Na}_2\text{CO}_3$ , stopper tightly, and macerate for at least 4 hours, with occasional shaking. Decant or filter rapidly a 50 cc aliquot of the clear supernatant liquid representing 5 g of the drug, transfer to a separator, and shake with 3 portions of 2%  $\text{H}_2\text{SO}_4$ , using 15, 10, 10 cc, etc., until the extraction is complete. Combine the acid solns in a separator, neutralize with  $\text{NH}_4\text{OH}$ , and add about 5 g of anhydrous  $\text{Na}_2\text{CO}_3$ , stirring until dissolved. Shake with 5 portions of ether, using 35, 30, 25, 20 and 15 cc, until extraction is complete, and combine the ether portions in a second separator. When clear, decant and filter into a small beaker thru a pledget of cotton previously wet with ether.

Evaporate the solvent to 5 cc volume on the steam bath with the aid of a fan, and add bromothymol blue indicator, VI, 108(e), and a measured excess of 0.02 *N*  $\text{H}_2\text{SO}_4$ . Cover with a watch-glass, return to the steam bath in order to dissolve any alkaloid adhering to the sides of the beaker, and then evaporate the ether. Titrate the excess acid with 0.02 *N* alkali. 1 cc of 0.02 *N* acid = 0.0033 g of ephedra alkaloids.

EPHEDRINE IN INHALANTS<sup>14</sup>—OFFICIAL, FIRST ACTION

## 42

## DETERMINATION

Weigh accurately into a small tared beaker, 5–10 g of the sample. Add 10 cc of 2%  $\text{H}_2\text{SO}_4$ , stir, and allow the mixture to stand about 15 min. Transfer to a small separator (automatic extractor optional), rinsing the beaker with small portions of ether. Shake gently, and transfer the acid layer to a second separator. Shake with 3 successive 10 cc portions of 2%  $\text{H}_2\text{SO}_4$ , rinsing the beaker with ether each time. Test for complete removal of alkaloid.

Neutralize the combined acid soln with  $\text{NH}_4\text{OH}$ , and add 5 cc in excess. Extract the soln with 30 cc of washed ether, transfer the aqueous layer to a second separator, and wash the ethereal extract with 1 cc of  $\text{H}_2\text{O}$ , adding the washings to the main aqueous soln. Swirl the ether in order to remove  $\text{H}_2\text{O}$  adhering to the side of the separator. After all the  $\text{H}_2\text{O}$  has been removed, filter the mixture into an Erlenmeyer flask thru a pledget of cotton wet with ether inserted in a small funnel. Repeat the extraction with liberal portions of washed ether at least 4 times, or until the alkaloid is removed completely, washing each portion with the same 1 cc of  $\text{H}_2\text{O}$ . Evaporate the ether to a volume of 10 cc on a steam bath with moderate heat by the aid of a current of air.

Remove from the bath. Add bromothymol blue indicator, VI, 108(e), and a measured excess of 0.02 *N* H<sub>2</sub>SO<sub>4</sub>. Add about 40 cc of CO<sub>2</sub>-free H<sub>2</sub>O, cover with a watch-glass, return to the steam bath in order to dissolve the alkaloid adhering to the sides of the flask, and evaporate all the ether. Titrate the excess acid with 0.02 *N* NaOH, using standard indicator, pH 6.0, for comparison. 1 cc of 0.02 *N* acid = 0.0033 g of ephedrine.

#### EPHEDRINE IN TABLETS—OFFICIAL, FIRST ACTION

43

##### PREPARATION OF SAMPLE

To determine the average weight per tablet, count and weigh 100 tablets or a number representative of the lot.

44

##### DETERMINATION

Weigh not less than 20 tablets. Powder in a mortar, weigh accurately a quantity equal to about 0.12 g of the alkaloidal salt, and transfer to a separator. Dissolve in the minimum quantity of H<sub>2</sub>O, then add 5 cc of NH<sub>4</sub>OH.

Extract the soln with 30 cc of washed ether. Transfer the aqueous layer to a second separator. Wash the ether extraction with 1 cc of H<sub>2</sub>O, adding the washings to the main aqueous soln. Swirl the ether in order to remove H<sub>2</sub>O adhering to the side of the separator. After all the H<sub>2</sub>O has been removed, filter into a beaker thru a pledget of cotton wet with ether inserted in a small funnel. Repeat the extraction with liberal portions of ether at least 4 times, or until the alkaloid is removed completely, washing each portion with 1 cc of H<sub>2</sub>O. Evaporate the ether to a volume of 10 cc on a steam bath with moderate heat before a fan and proceed as directed under 42, beginning with "Remove from the bath. Add . . ." to end of paragraph. 1 cc of 0.02 *N* acid = 0.0043 g of ephedrine hydrochloride, 0.00428 g of ephedrine sulfate, and 0.0033 g of ephedrine.

#### METHENAMINE (HEXAMETHYLENETETRAMINE) IN TABLETS—TENTATIVE

45

##### REAGENT

*Modified Nessler's reagent.*—(1) Dissolve 10 g of HgCl<sub>2</sub>, 30 g of KI, and 5 g of acacia in 200 cc of H<sub>2</sub>O, and filter thru a pledget of cotton; (2) dissolve 15 g of NaOH in 100 cc of H<sub>2</sub>O; (3) mix 20 cc of soln (1) with 10 cc of soln (2).

46

##### PREPARATION OF SAMPLE

Ascertain the weight of 20 or more tablets, triturate in a mortar to a fine powder, and keep in a small capsule tightly closed with a cork or glass stopper.

47

##### DETERMINATION

Weigh 0.5 g of the powdered product on a metal scoop or watch-glass, transfer to a round-bottomed flask, and add H<sub>2</sub>O to a total volume of 100 cc and finally 25 cc of HCl (1+2.5). Connect with a reflux condenser (preferably of the worm type) and boil gently for 15 min. Cool, wash the condenser tube with a little H<sub>2</sub>O, and transfer the contents of the flask quantitatively to a 250 cc volumetric flask, finally diluting to the mark with H<sub>2</sub>O. Chill 30 cc of the modified Nessler's reagent, 45, and add a 10 cc aliquot of the hydrolyzed soln of the sample. Wash down the neck of the container with a jet of H<sub>2</sub>O and allow to stand for at least 1 min. Add 10 cc of acetic acid (1+1.5) in such a manner that the inside of the neck is completely washed by the reagent, mix quickly and thoroly by rotating and tilting the flask, and immedi-

ately add from a buret 20 cc of 0.1 *N* I soln. Titrate the excess I with 0.1 *N*  $\text{Na}_2\text{S}_2\text{O}_3$  soln, adding 5–10 drops of starch indicator, VI, 3(e), toward the end of the operation, to the disappearance of the blue coloration. The final color of the soln is a pale straw-green. If preferred, the end point may be determined by the reappearance of a faint blue coloration by the addition of a drop of the I soln. 1 cc of 0.1 *N* I soln = 0.001168 g of methenamine.

METHYLENE BLUE (METHYLTHIONINE CHLORIDE)<sup>12</sup>—OFFICIAL

48

## PREPARATION OF SAMPLE

(a) *Tablets*.—Weigh separately at least 20 tablets to ascertain the variation in weight. Weigh collectively all unbroken tablets and calculate the average weight per tablet. Powder finely in a mortar at least 10 tablets or 5 g of methylene blue and protect from moisture in a weighing bottle.

(b) *Capsules*.—Count and weigh a representative number of capsules and ascertain the gross weight per capsule. Open the capsules and transfer as much as possible of the contents to a weighing bottle. Deduct the weight of the cleaned, empty capsules from the gross weight and calculate the average net contents. To clean the gelatine capsules cut in two if necessary and wash by agitating with alternate portions of alcohol and ether. Repeat until thoroughly clean, finally removing the ether before a fan or air blast. A few drops of glacial acetic acid mixed with the alcohol aids in the cleaning.

49

## PREPARATION OF SOLUTION

(a) *Foreign material absent*.—Weigh into a 50 cc beaker 0.1–0.14 g of the prepared sample, 48, and transfer to a 200 cc volumetric flask with 100–140 cc of  $\text{H}_2\text{O}$ . Dissolve completely by heating on a steam bath, with frequent shaking, for 30 min.

(b) *Oils or water-insoluble material present*.—Transfer to a 150 cc beaker a weighed quantity of the prepared sample, 48, corresponding to 0.1–0.14 g of methylene blue. Add 15 cc of  $\text{CCl}_4$ , warm on a steam bath a few min., and stir with a glass rod to dissolve the oils. Transfer to a 100 cc separator, using about 50 cc of hot  $\text{H}_2\text{O}$  and a little  $\text{CCl}_4$  if necessary. Cool, shake, and allow to separate. Transfer the  $\text{CCl}_4$  with the undissolved material into a second separator for further treatment. (A clear aqueous soln of the dye should now remain in the first separator. If not clear, extract with another 15 cc portion of  $\text{CCl}_4$ , transferring in a similar manner any remaining insoluble material to the second separator.) Add about 10 cc of  $\text{CCl}_4$  to the second separator and remove the methylene blue by shaking vigorously with 20–40 cc portions of  $\text{H}_2\text{O}$  until practically no more dye is extracted. (A few drops of glacial acetic acid hastens this extraction.) To the aqueous extracts in a 400 cc beaker add the main soln from the first separator, cover with an inverted watch-glass on glass rods, and evaporate to a volume of about 50 cc. Proceed as directed under (c). The  $\text{CCl}_4$  soln may be reserved for qualitative tests for oils.

(c) *Water-soluble material present*.—Use either the aqueous soln from (b), or a weighed portion of the sample corresponding to 0.1–0.14 g of methylene blue. Dissolve completely by heating on a steam bath in a 150 cc beaker with about 50 cc of  $\text{H}_2\text{O}$  for 30 min., shaking occasionally. Transfer to a 100 cc separator, keeping the volume as small as possible. Extract with dichlorhydrin, using 10, 5, 3, and 2 cc portions. Combine the dichlorhydrin extracts in a 200–300 cc separator, add 3 or 4 times their volume of  $\text{CCl}_4$ , and extract the dye with  $\text{H}_2\text{O}$  by repeated vigorous shaking with 30–50 cc portions. A few drops of glacial acetic acid hastens the re-

moval. From the combined aqueous extracts remove any traces of dichlorhydrin by shaking once with about 15 cc of  $\text{CCl}_4$ , which is drawn off after settling for 5-10 min. Evaporate the aqueous extracts to about 50 cc over a flame, covering the beaker as in (b) with an inverted watch-glass. Transfer to a 200 cc volumetric flask. Dissolve completely by heating on a steam bath with frequent shaking for 30 min.

## 50

## DETERMINATION

Conduct a blank in the same manner as the determination, including the filtration. Cool the soln from 49(a) or (c), add 50 cc of glacial acetic acid, shake thoroly, and allow to stand at least 25 min. Add from a buret a total of 30 cc of 0.2 *N* I soln, adding the first 10 cc by fast drops with constant rotating of the flask and the remaining 20 cc at full speed, and continue the shaking. Stopper the flask and allow to stand 50 min., shaking thoroly 5 or 6 times during the interval. Dilute to the mark with  $\text{H}_2\text{O}$ , shake, and let stand 10 min. longer. Filter rapidly thru a dry, folded, 12 cm filter paper. Titrate a 100 cc aliquot with 0.1 *N*  $\text{Na}_2\text{S}_2\text{O}_8$  soln with or without starch indicator, as desired. Correct for the number of cc required to titrate the blank run in the same way. 1 cc of 0.2 *N* I soln = 0.01495 g of methylene blue ( $\text{C}_{16}\text{H}_{18}\text{N}_2\text{ClS} \cdot 3\text{H}_2\text{O}$ ).

## 51

## CAMPHOR—OFFICIAL

Weigh accurately into a 400 cc round-bottomed Pyrex flask, a sufficient quantity of the powdered material to contain approximately 2 g of camphor. Add 10 cc of benzol and 10 cc of  $\text{H}_2\text{O}$  and connect the flask with an apparatus for steam distillation. Use an 8-12 in. bulb condenser, well cooled, the outlet of which reaches to the bottom of a 200 cc flask. Distil with steam, collecting the benzol and about 100 cc of aqueous distillate. Disconnect the condenser and wash it slowly with 5 cc of alcohol from a pipet in such a manner as entirely to wet the inside of the condenser. Wash the condenser in the same manner with 10 cc of benzol. Add both washings to the contents of the receiver. Saturate the distillate with  $\text{NaCl}$ , add sufficient  $\text{H}_2\text{SO}_4$  (1+9) to insure acidity, transfer to a separator, shake, and separate the two layers. Rinse the original receiver with 10 cc of benzol and with the rinsings re-extract the aqueous soln. Separate the aqueous layer and extract it once more with 10 cc of benzol. Wash the combined benzol portions with 10 cc of saturated salt soln rendered distinctly alkaline with  $\text{Na}_2\text{CO}_3$ . Separate the layers and extract the aqueous layer with 10 cc of benzol. Discard the aqueous solns, transfer the benzol portions to a 50 cc volumetric flask, and make up to the mark with benzol. Shake the soln and filter it into a 200 mm polariscope tube, using a water-jacketed tube, if necessary, in order to maintain a constant temp. of  $20^\circ$ . Make 10 readings, using a bichromate filter, and take the average reading for calculating the camphor. Calculate the quantity of camphor (*Q*) contained in the 50 cc of benzol and, therefore, in the sample taken, from the average reading in circular degrees (*a*) by the following formula:

$$Q = 0.6171a - 0.0022a^2.$$

The value of *Q* does not vary directly with the length of the tube. If a longer or shorter tube than directed is used, correct the value of *a* to a 200 mm tube, and then make the calculation by the above formula.<sup>24</sup>

## MONOBROMATED CAMPHOR IN TABLETS

*Method I.<sup>26</sup>—Official*

52

## REAGENT

*Sodium amalgam.*—Cut about 1 g of bright metallic Na into small pieces and dissolve in 100 g of warm Hg contained in a small porcelain mortar by impaling the pieces successively on the point of a file and holding them submerged in the Hg until the rather violent action is complete. Keep the resulting amalgam in a tightly corked bottle.

53

## PREPARATION OF SAMPLE

Count and weigh a suitable number of tablets to ascertain the average weight; reduce them to a fine powder and keep in a tightly stoppered bottle.

54

## DETERMINATION

Weigh a portion of the powdered sample corresponding to 0.1–0.2 g of monobromated camphor, and transfer quantitatively with 20 cc of alcohol and 10 cc of H<sub>2</sub>O to a small (100 cc) round-bottomed flask containing 15 g of the Na amalgam. Connect the flask by means of a rubber stopper with a vertical condenser. Boil the mixture gently over a wire gauze at least 30 min. Cool slightly and wash out the condenser tube with 5 cc of alcohol and 5 cc of H<sub>2</sub>O, receiving the washings in the flask. Place the flask on a steam bath and heat for another hour, or until the evolution of H<sub>2</sub> has nearly or quite ceased. Toward the latter part of this operation, to facilitate the reduction, render the liquid about neutral with a few drops of acetic acid. Transfer the contents of the flask to a separator, preferably of the Squibb type, withdrawing the Hg into a second separator and washing it with at least two 50 cc portions of H<sub>2</sub>O. Pass the several aqueous solns quantitatively thru a small filter, collecting the clear filtrate in a suitable beaker. Precipitate with 10% AgNO<sub>3</sub> soln, add about 5 cc of HNO<sub>3</sub>, and filter, collecting the AgBr on a weighed Gooch crucible. Wash with H<sub>2</sub>O and alcohol, dry at 100°, and weigh. The weight of AgBr  $\times 1.23$  = the quantity of monobromated camphor originally present in the portion taken for analysis. Run a control on the amalgam to determine whether any correction is necessary.

*Method II.<sup>26</sup>—Tentative*

55

## PREPARATION OF SAMPLE

Proceed as directed under 53.

56

## DETERMINATION

Weigh in a small beaker a quantity of the powdered sample equivalent to about 0.2 g of monobromated camphor, add 25 cc of alcohol, warm on a steam bath, and filter into a flask (preferably about 250 cc capacity and provided with a ground-in condenser), washing both beaker and filter with warm alcohol. Add 50 cc of alcoholic KOH soln, **XXXI**, **22**, and 25 cc of alcoholic AgNO<sub>3</sub> soln (0.2 g in 50 cc of alcohol), and connect with the reflux condenser. Boil gently 1.5 hours, adding at intervals thru the condenser 25 cc more of the alcoholic AgNO<sub>3</sub> soln. Cool, and transfer the contents of the flask to a large evaporating dish. Dilute to 200 cc and decant into a beaker, washing the sediment of Ag<sub>2</sub>O with H<sub>2</sub>O by decantation. Boil the soln 5 min. with 1 g of Zn dust to clarify; filter into another beaker, washing thoroughly with H<sub>2</sub>O, and add HNO<sub>3</sub> to decided acidity and 0.1 N aqueous AgNO<sub>3</sub> soln to complete precipitation. When the AgBr has agglutinated filter on a weighed Gooch

crucible, wash with  $H_2O$  and alcohol, dry at  $100^\circ$ , and weigh. Weight of  $AgBr \times 1.23$  = the quantity of monobromated camphor originally present in the sample taken for analysis. Run a control on the reagents used. Correct for the presence of halogens if necessary.

#### NITROGLYCERIN<sup>27</sup>—OFFICIAL, FIRST ACTION

57

##### REAGENT

*Alcoholic potassium hydroxide.*—Dissolve 15 g of KOH in ethyl alcohol and dilute with alcohol to 100 cc.

58

##### APPARATUS

(a) *Connecting bulb.*—Hopkins style, about 7.6 cm (3 in.) in diameter. This style has a long inlet tube with an opening on the side of the tube.

(b) *Condenser.*—Water-cooled, length about 56 cm (22 in.), and preferably of Pyrex glass.

(c) *Adapter tube.*—About 2.25 cm ( $\frac{1}{4}$  in.) in diameter at the top and with narrow outlet.

(d) *Scrubber-trap.*—Any efficient trap in which all the vapor is washed thoroly with  $H_2O$  before it leaves the distilling flask. (See Fig. 48.)

#### DETERMINATION

59

##### Method I.

(a) Place in a 50 cc beaker a sufficient quantity of the weighed sample to yield about 0.0324 g of nitroglycerin. If the sample consists of tablets, count those taken;

if of powdered material, mix thoroly before weighing. Add 10 cc of ether, and to facilitate extraction reduce the tablets to a fine powder by means of a glass stirring rod having a flattened end. Stir thoroly. Decant the ether thru a dry 7 cm quantitative filter paper into a 250 cc beaker containing 10 cc of alcohol. Hold the filter paper in place in the funnel with the stirring rod and pour the ether down the rod. Make four additional extractions in the same way. Dissolve the ether-insoluble residue in a small quantity of  $H_2O$ , transfer the soln to a separator, and extract it twice with 10 cc portions of ether. Filter these extracts, add them to the first extracts, and evaporate the combined solns to a volume of about 10 cc by means of a fan.

(b) Transfer the alcoholic soln containing the nitroglycerin to an 800 cc Kjeldahl flask, rinsing the beaker first with 10 cc of alcohol and then with a little  $H_2O$ . Dilute to about 300 cc with recently boiled and cooled  $NH_3$ -free  $H_2O$  and place the flask on a wire gauze with an asbestos center. Add 2 g of Devarda alloy (by means of a funnel), about 4 cm of heavy (about 16 gage) Al wire, and 10–15 cc of the alcoholic KOH soln. Immediately after adding the alkali, place a little  $H_2O$  in the scrubber trap and insert into the flask the rubber stopper carrying the connecting bulb and trap. Connect the outlet tube of the connecting bulb with the water-cooled condenser, which has been fixed in an upright position fitted with

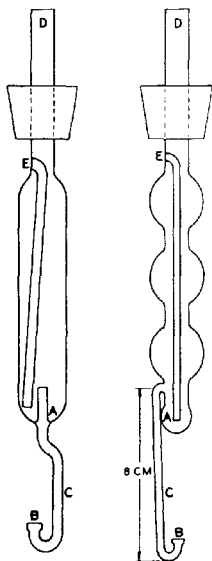


FIG. 48.—SCRUBBER TRAP FOR AMMONIA DISTILLATION

the adapter dipping to the bottom of a 500 cc Erlenmeyer flask containing a measured volume (about 25 cc) of 0.02 *N* acid (HCl or H<sub>2</sub>SO<sub>4</sub>) and 10–15 cc of H<sub>2</sub>O, and inclined in such a way that the tip of the adapter is submerged as far as practicable under the surface of the liquid in the flask. Heat the distillation flask for about 1 hour, using a small flame and regulating the heat applied so that rapid evolution of H— but no appreciable distillation—takes place. Gradually increase the heat until distillation begins; when active foaming ceases, continue the distillation with a large flame until all but about 40 cc of the liquid in the distilling flask has distilled over. Lower the flame toward the end of the distillation to avoid cracking the flask. Remove the receiver containing the distillate, add sufficient methyl red indicator, II, 55(a), to make the soln red, and titrate the excess of acid with 0.02 *N* NaOH soln. From the difference between this excess and the quantity added, after making such correction as may be shown to be necessary by a blank test with the same quantity of reagents and distilled in the same manner, calculate the percentage of nitroglycerin in the sample. 1 cc of 0.02 *N* acid neutralized by the ammonia = 0.001514 g of nitroglycerin.

60

*Method II.*

Place in a glass-stoppered Erlenmeyer flask a sufficient quantity of the sample, accurately weighed, to yield about 0.0648 g (1 grain) of nitroglycerin. If the sample consists of tablets, count those taken; if of powdered material, mix thoroly before weighing the portion taken for analysis. Add 50 cc of alcohol by means of a pipet. To facilitate extraction reduce the tablets to a fine powder with a glass stirring rod flattened at one end. Stopper the flask and shake. Allow the mixture to settle, transfer a 25 cc aliquot of the clear soln to an 800 cc Kjeldahl distilling flask, dilute to about 300 cc with the NH<sub>3</sub>-free H<sub>2</sub>O, and proceed as directed in 59(b).

ELIXIR OF TERPIN HYDRATE<sup>28</sup>—TENTATIVE

61

## REAGENTS

(a) *Salt soln.*—Dissolve 20 g of common salt in H<sub>2</sub>O and make to 100 cc, or dilute 3 volumes of saturated salt soln with 1 volume of H<sub>2</sub>O.

(b) *Alcohol-chloroform soln.*—CHCl<sub>3</sub> containing 5–7% by volume of alcohol.

62

## DETERMINATION

Measure 10 cc of the sample into a separator and dilute with 25 cc of the salt soln. (The quantity to be weighed should be approximately 0.2 g, and the dilution with salt soln should be such as to reduce the alcoholic content to about 10–15% by volume before extraction with alcohol-chloroform.) Extract successively with six 15 cc portions of alcohol-chloroform, separating the CHCl<sub>3</sub> layer carefully each time so that none of the watery layer will be carried thru with the CHCl<sub>3</sub>. Collect all the CHCl<sub>3</sub> fractions and wash twice with the salt soln, using 15 cc for the first washing and 5 cc for the second. Wash each salt washing with a 5 cc portion of alcohol-chloroform, adding this portion to the original CHCl<sub>3</sub> extracts. Filter the combined CHCl<sub>3</sub> extracts containing the terpin hydrate in soln thru a plug of cotton into a 100 cc low-form, tared beaker, being sure that the separation from salt wash water is perfect. Evaporate off the CHCl<sub>3</sub> at room temp. with the aid of an air blast. Use no heat in the evaporation. Wipe off the beaker when the CHCl<sub>3</sub> is entirely gone, allow to stand in the balance for 10 min., and weigh. Do not dry in a desiccator because terpin hydrate loses H<sub>2</sub>O under these conditions. Report as g per 100 cc.



## OPIUM ALKALOIDS

MORPHINE IN TABLETS<sup>21</sup>

63

*Qualitative Tests—Official*

(1) To the residue or tablet add  $\text{HNO}_3$ . An orange-red color fading to yellow is produced.

(2) To an aqueous soln add a few drops of a 10%  $\text{K}_3\text{Fe}(\text{CN})_6$  soln and then a drop of a 10%  $\text{FeCl}_3$  soln. A deep blue soln results; a blue precipitate separates on standing.

(3) See Microchemical Tests, 179.

*Quantitative Method—Official*

64

## REAGENT

*Alkaline salt soln.*—Dissolve 30 g of  $\text{NaOH}$  in  $\text{H}_2\text{O}$ , dilute to 1 liter, add  $\text{NaCl}$  to saturation, and filter.

65

## PREPARATION OF SAMPLE

To ascertain variation in weight, weigh separately at least 20 tablets. Also weigh collectively a representative number of unbroken tablets and calculate the average weight per tablet. To insure representative sampling in tablets containing more than  $\frac{1}{4}$  grain of alkaloid, pulverize about 20 tablets, mix the powder thoroly, and protect it from moisture in a weighing bottle.

66

## DETERMINATION

Transfer to a small separator a sufficient number of the tablets, or powdered material equal to a multiple of the average weight per tablet, to represent approximately 0.15 g of the alkaloid. Moisten with 5 cc of  $\text{H}_2\text{O}$ , shake gently, and then dissolve completely by adding 10 cc of the alkaline salt soln. To the alkaline salt soln, add a small piece of litmus paper and then  $\text{HCl}$ , dropwise, until it is neutral. Add 10 drops in excess. Add 5 cc of alcohol, carefully neutralize with  $\text{NH}_4\text{OH}$  dropwise, and then add 5 drops in excess. Invert the separator and open the stopcock to insure neutralization of residual acid. Immediately extract, at least 6 times, with  $\text{CHCl}_3$ -alcohol solvent (90+10), using 30, 20, 20, 10, 10, and 5 cc, or until the alkaloid is completely removed. Test for the complete extraction of the alkaloid. Make an additional extraction with 10 cc of the  $\text{CHCl}_3$ -alcohol solvent, evaporate the solvent in a separate beaker, dissolve the residue in a few drops of methyl alcohol, add a drop of methyl red, II, 55(a), and dilute with 20 cc of  $\text{H}_2\text{O}$ , carbonate free. (A yellow color indicates incomplete extraction.) Titrate, and add the quantity thus obtained to the total. Combine the  $\text{CHCl}_3$ -alcohol extractions in a second separator, into the stem of which is inserted a pledget of cotton wet with  $\text{CHCl}_3$ . Wash the combined extractions with 1 cc of  $\text{H}_2\text{O}$ . When clear, filter into a small beaker. Extract the wash  $\text{H}_2\text{O}$  twice with small portions of the  $\text{CHCl}_3$ -alcohol solvent. Evaporate on a water bath, using an electric fan to prevent decrepitation of the residue. When dry, remove immediately and complete the determination by one of the following procedures:

(1) To the alkaloidal residue add 2-3 cc of methyl alcohol, cover the beaker with a watch-glass, and heat on a steam bath until the residue, including any portions thereof that may adhere to the upper part of the beaker, is completely dissolved. Add 2 drops of the methyl red indicator and, without dilution with  $\text{H}_2\text{O}$ , titrate

carefully with 0.02 *N* H<sub>2</sub>SO<sub>4</sub> to a faint pink, avoiding an excess. Cover the beaker and digest on a steam bath until all particles are completely dissolved. If more than 2 cc of alcohol is added, evaporate the excess. Cool, and dilute with 50 cc of boiled H<sub>2</sub>O. (The soln should now be yellow.) Finish the titration with the standard acid to a faint red.

(2) Dissolve the residue in 2-3 cc of methyl alcohol on a steam bath. Add 2 drops of the methyl red indicator and then add from a buret 5-10 cc excess of 0.02 *N* H<sub>2</sub>SO<sub>4</sub>, noting the total quantity used. Cover the beaker with a watch-glass and heat on a steam bath until the residue, including any portions thereof that may adhere to the upper part of the beaker, is completely dissolved. Dilute with 50 cc of cold, previously boiled H<sub>2</sub>O. Titrate back with the 0.02 *N* NaOH soln. The H<sub>2</sub>O and alkali should be sufficiently free from carbonates to insure a sharp end point with methyl red. 1 cc of 0.02 *N* acid = 0.007513 g of morphine hydrochloride, C<sub>17</sub>H<sub>19</sub>O<sub>3</sub>NHCl · 3H<sub>2</sub>O, or 0.007585 g of morphine sulfate, (C<sub>17</sub>H<sub>19</sub>O<sub>3</sub>N)<sub>2</sub>H<sub>2</sub>SO<sub>4</sub> · 5H<sub>2</sub>O.

Alkaloids other than morphine are extracted by CHCl<sub>3</sub>, while morphine remains in the fixed alkali soln. In general, this separation is unnecessary. If the tablets are of unknown composition or atropine or scopolamine is present, shake the alkaline salt soln with 10 cc portions of washed CHCl<sub>3</sub> (use ether for the separation of atropine). Transfer the clear solvent to a small beaker and evaporate on a steam bath. If a residue is obtained, apply the usual tests.

## 67

APOMORPHINE IN TABLETS<sup>20</sup>—OFFICIAL, FIRST ACTION

Weigh a number of tablets equivalent to about 0.065 g (1 grain) of the alkaloid or of its salt and dissolve in 10 cc of H<sub>2</sub>O in a separator. Add 1 cc of a freshly prepared saturated soln of NaHCO<sub>3</sub> and 25 cc of peroxide-free ether, and shake the mixture. After separation, draw off the lower layer into a second separator and transfer the ethereal layer to a third separator. Extract the mixture in the second separator repeatedly with 15 cc portions of ether until the alkaloid has been completely removed, using the second and first separators alternately for the shaking, and collecting all the ethereal soln in the third. Discard the aqueous soln. Wash the ethereal soln of the alkaloid 3 times with 5 cc portions of H<sub>2</sub>O, uniting the aqueous washings in a clean separator. Extract these washings with a little fresh peroxide-free ether. Discard the aqueous portion, wash the ether with H<sub>2</sub>O, discard the washings, and add the washed ether to the main portion of the ethereal soln. Add 20 cc of 0.02 *N* H<sub>2</sub>SO<sub>4</sub> to the ethereal soln of the alkaloid in the separator and shake the mixture thoroly. Transfer the mixture to a beaker, wash the separator twice with 5 cc portions of H<sub>2</sub>O, adding the washings to the acid liquid in the beaker, and without delay evaporate the ether at a low temp., preferably on the water bath with the aid of a blast of air. Titrate the excess of acid with 0.02 *N* NaOH, using one drop of methyl red indicator, II, 55(a). 1 cc of 0.02 *N* H<sub>2</sub>SO<sub>4</sub> = 0.00625 g of apomorphine hydrochloride, C<sub>17</sub>H<sub>17</sub>O<sub>2</sub>NHCl +  $\frac{1}{2}$ H<sub>2</sub>O.

CODEINE IN TABLETS<sup>20</sup>

## 68

## Qualitative Tests—Official

(a) To the residue or tablet add HNO<sub>3</sub>. A yellow color is produced.

(b) To 3 cc of an aqueous soln (1+200), add a few drops of a 10% K<sub>3</sub>Fe(CN)<sub>6</sub> soln and 1 or 2 drops of FeCl<sub>3</sub> soln. A green color is produced.

## 69

## Quantitative Method—Official

Transfer to a small separator a sufficient number of tablets, or powdered material,

65, equal to a multiple of the average weight per tablet, to represent approximately 0.15 g of the alkaloid. Dissolve in a minimum of  $\text{H}_2\text{O}$ , not to exceed 5 cc, acidified with 2 drops of  $\text{HCl}$ . Add solid  $\text{NaHCO}_3$  until neutralized, then a slight excess, and extract 5 times with  $\text{CHCl}_3$ , using 30, 20, 20, 10, and 5 cc. Test for complete extraction of the alkaloid. Make an additional extraction with 10 cc of  $\text{CHCl}_3$ ; evaporate the solvent in a separate beaker; dissolve the residue in a few drops of methyl alcohol; add a drop of methyl red indicator, II, 55(a); and dilute with 20 cc of  $\text{H}_2\text{O}$ , carbonate free. A yellow color indicates incomplete extraction. Titrate, and add the quantity thus obtained to the total. Combine the  $\text{CHCl}_3$  extractions in a second separator, into the stem of which is inserted a pledget of cotton wet with  $\text{CHCl}_3$ . Wash the combined extractions with 1 cc of  $\text{H}_2\text{O}$  containing 1 drop of  $\text{NH}_4\text{OH}$  and proceed as directed under 66, beginning with "Evaporate on a water bath." 1 cc of 0.02 N  $\text{H}_2\text{SO}_4 = 0.00787$  g of codeine sulfate,  $(\text{C}_{18}\text{H}_{21}\text{O}_3\text{N}_2)_2\text{H}_2\text{SO}_4 \cdot 5\text{H}_2\text{O}$ , or to 0.00849 g of codeine phosphate,  $\text{C}_{18}\text{H}_{21}\text{O}_3\text{N}_2\text{H}_2\text{PO}_4 \cdot 1\frac{1}{2}\text{H}_2\text{O}$ .

#### DIACETYLMORPHINE (HEROIN) IN TABLETS<sup>2</sup>

70

##### *Qualitative Test—Official*

Heat about 0.1 g with 1 cc of  $\text{H}_2\text{SO}_4$  and 1 cc of alcohol. Ethyl acetate, readily recognized by its odor, is formed.

71

##### *Quantitative Method—Official*

Weigh and transfer directly to a small separator a number of tablets representing approximately 0.15 g of diacetylmorphine. Dissolve in 5 cc of  $\text{H}_2\text{O}$  containing 1 drop of acetic acid. Add 1 cc of  $\text{NH}_4\text{OH}$  and extract 5 times with  $\text{CHCl}_3$ , using 30, 20, 10, 10, and 5 cc, respectively. Combine the  $\text{CHCl}_3$  extracts in a second separator, into the stem of which is inserted a pledget of cotton wet with  $\text{CHCl}_3$ . Wash the combined extraction with 1 cc of  $\text{H}_2\text{O}$  and proceed as directed under 66, beginning with "Evaporate on a water bath." 1 cc of 0.02 N  $\text{H}_2\text{SO}_4 = 0.008473$  g of diacetylmorphine hydrochloride,  $\text{C}_{21}\text{H}_{23}\text{O}_3\text{NHCl} \cdot \text{H}_2\text{O}$ .

#### IPOMEA<sup>23</sup>—TENTATIVE

72

##### DETERMINATION OF RESIN

Place 10 g of the drug in a No. 60 powder in an Erlenmeyer flask of about 250 cc capacity and add 50 cc of alcohol. Fit the flask with a stopper thru which is inserted a glass tube about 1 m long to act as a condenser, and heat the mixture on a gently simmering steam bath for 30 min., shaking occasionally. Transfer the contents of the flask to a small percolator and percolate slowly with warm alcohol until about 95 cc of tincture has been obtained. To ascertain whether extraction is complete, collect a further 10 cc of percolate and pour a few drops into cold  $\text{H}_2\text{O}$ ; if more than a faint cloudiness appears, continue the percolation with warm alcohol until the test for resin fails. Concentrate the additional percolate by evaporation and add the residue to the flask before making up to volume. Cool the percolate to room temp. and make up the soln to 100 cc with alcohol. Mix well.

Evaporate 25 cc of the prepared tincture (representing 2.5 g of drug) to dryness on the water bath in a beaker or flask of suitable size and dry the residue until it is free from alcohol. Add 15 cc of  $\text{H}_2\text{O}$ , bring the mixture to boiling, allow to cool about 3 min., and stir well with a flat-headed glass rod for 2 min. to insure thoro washing of the resin. Cool the mixture by placing the container in a jar of ice-cold  $\text{H}_2\text{O}$  and decant the wash  $\text{H}_2\text{O}$  onto a 9 cm filter paper. Repeat the washing of the resin with

another 15 cc portion of  $H_2O$ , boiling and cooling the mixture, kneading the resin as before, and decanting the washings onto the filter, as described previously. Repeat the washing and kneading process with hot  $H_2O$  a third time. Dissolve the residue in the container in 10 cc of warm alcohol and pour the soln onto the filter, collecting the filtrate in a weighed beaker or flask. Use sufficient hot alcohol in small portions to completely transfer the soln of the resin to the filter and insure thoro washing of the filter. Evaporate the combined filtrate and washings to apparent dryness, add 1 cc of absolute alcohol, and evaporate the solvent, taking care to rotate the container in an inclined position as the last portions of the solvent are dissipated. Dry the residue at  $80^\circ$  to constant weight.

73

JALAP<sup>22</sup>—TENTATIVE

Proceed as directed under 72.

74

PODOPHYLLUM<sup>23</sup>—TENTATIVE

## DETERMINATION OF RESIN

Place 10 g of the sample in a No. 60 powder in an Erlenmeyer flask of about 250 cc capacity and add 35 cc of alcohol. Fit the flask with a stopper thru which is inserted a glass tube about 1 m long to act as a condenser, and place the flask on a gently simmering steam bath for 30 min., shaking occasionally. Transfer the contents of the flask to a small percolator and percolate slowly with hot alcohol until about 95 cc of percolate has been obtained. Collect about 10 cc more of the percolate in a separate container. Cool the first percolate to room temp. and make up the volume to 100 cc with a portion of the second percolate.

Place 50 cc of the alcoholic soln in a tared beaker and add 2 cc of  $H_2O$ . Evaporate until the percolate weighs 3 g. If the weight should fall below 3 g, add alcohol dropwise to make up to 3 g. Pour the residue slowly, with constant stirring, into a second beaker containing 10 cc of  $H_2O$  previously mixed with 1 cc of normal HCl and cooled to a temp. below  $10^\circ$ . (Pellets of ice placed in the beaker and renewed from time to time serve well.) Add 5 cc of  $H_2O$  and a few drops of 10% HCl to the tared beaker, stir well, and rub the sides of the container with a glass rod. Add the mixture to the second beaker and allow to stand overnight in a refrigerator. Decant the supernatant liquid into a tared Gooch crucible and transfer the precipitate to the crucible by means of small portions of cold  $H_2O$  slightly acidulated with HCl. (If preferred, collect the precipitate on a filter paper and, after washing, dissolve in hot alcohol, collecting the soln in the tared beaker.) Dry the contents of the crucible at  $80^\circ$  and weigh. If particles of resin adhere to either beaker, dissolve them in alcohol, evaporate the solvent in the tared beaker, and dry the residue at  $80^\circ$ . Cool, weigh, and add the total net weight to the weight of the contents of the crucible.

75

ALOID<sup>24</sup>—TENTATIVE

(Applicable to mixtures containing cascara, rhubarb, senna, and other acid hydrolyzable anthraglucosides, as well as to resins and phenolphthalein, with aloin or aloes.)

Dry a sufficient quantity of the powdered material for 1 hour at  $110^\circ$  (or of a dealcoholized soln if a liquid) to insure approximately 0.3 g of aloin. Add 10 cc of  $H_2O$  and a few cc of 5% NaOH soln. Transfer the mixture to a 100 cc volumetric flask, dilute to about 75 cc and make acid with  $H_2SO_4$ , working rapidly as aloin is attacked by alkali. Dilute to mark, and add a few glass beads if much undissolved material is present. Shake occasionally during an hour to insure soln of aloin. Filter.

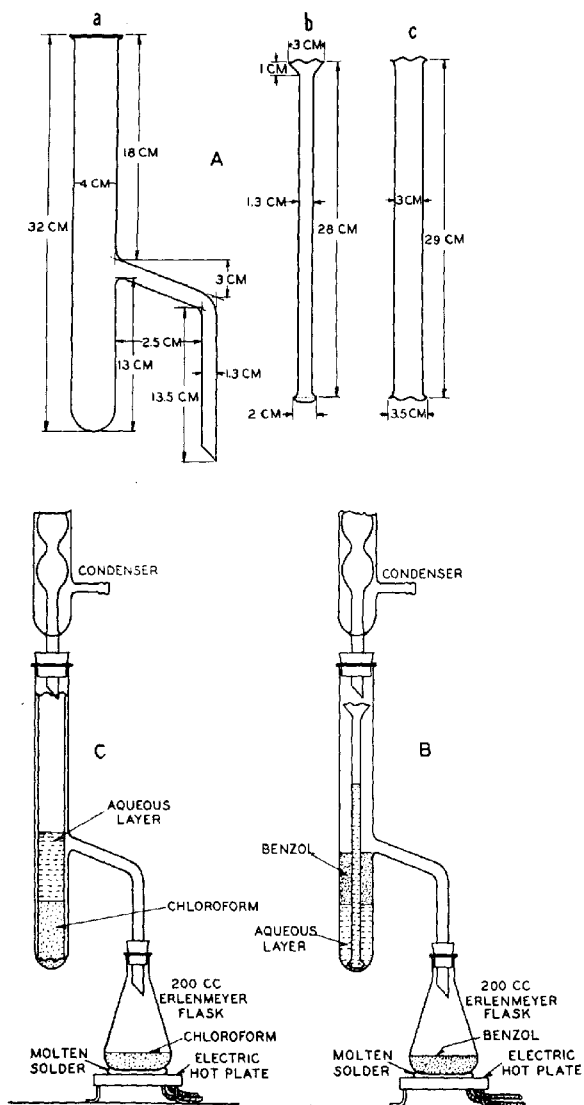


FIG. 49.—CONTINUOUS EXTRACTION APPARATUS

and transfer a 40 cc aliquot to which has been added 10 cc of 10%  $\text{H}_2\text{SO}_4$  (by weight) to a continuous extraction apparatus which has been previously charged with  $\text{CHCl}_3$  (A, Fig. 49). Reflux to exhaustion (about 2 hours). Disconnect the apparatus and transfer all the aqueous soln to a separator, discarding the  $\text{CHCl}_3$ . Saturate the soln with salt and shake out with 30 cc portions of  $\text{CHCl}_3$ -alcohol mixture (3+1). Test for complete removal of aloin by evaporating a portion of the 6th extraction. (More extraction may be necessary.) Shake violently.

Combine the extracts and wash with 1 cc of  $\text{H}_2\text{O}$ , to which is added 1 g of sodium acid carbonate, or more if necessary to insure an excess. Filter, evaporate, add 5 cc of  $\text{CHCl}_3$ , evaporate, dry at  $110^\circ$  for 1 hour, cool, and weigh rapidly. Weight = aloin in aliquot taken.

As a check, acetylate the aloin. This may be done by dissolving in acetic anhydride (about 10 cc), adding an excess (about 2 g) of powdered anhydrous sodium acetate and boiling for 5 min. in an acetylation flask placed in an oil bath. Wash sample from flask with additional acetic anhydride and evaporate to apparent dryness in a hood with a good draft. Add 10 cc of  $\text{H}_2\text{O}$  and heat for several minutes. Transfer with the aid of  $\text{CHCl}_3$  to a separator, washing the flask with successive portions of  $\text{CHCl}_3$ , and shake out with two additional 10 cc portions of  $\text{CHCl}_3$  (the aloin hexaacetate formed is soluble in  $\text{CHCl}_3$ ). Combine and filter, evaporate, add 10 cc of  $\text{CHCl}_3$ , evaporate, dry at  $110^\circ$  for 1 hour, cool, and weigh. Weight  $\times 0.615$  = aloin.

#### PHENOLPHTHALEIN IN TABLETS

##### *Ether Extraction Method<sup>28</sup>—Official*

(Not applicable in the presence of other ether extractives.)

76

#### PREPARATION OF SAMPLE

Count and weigh a number of tablets to ascertain the average weight, grind to a fine powder, and mix thoroly.

77

#### DETERMINATION

Weigh a portion of the powdered material representing about 0.1 g of phenolphthalein, transfer to a separator by means of 10 cc of 5%  $\text{NaOH}$  soln and a little  $\text{H}_2\text{O}$ , and extract 3 or 4 times with ether, using 25 cc for the first and 20 cc for each subsequent extraction. Transfer the ether extracts to a second separator and wash twice with 5 cc of the 5%  $\text{NaOH}$  soln. (Substances like quinine, acetanilid, acetphenetidin, as well as any unsaponified fatty material or mineral oil, if present, will be removed by extraction with ether.) Combine the alkaline solns and acidify with  $\text{HCl}$ . Extract with ether as before, until all the phenolphthalein has been removed, as determined by testing a portion of the ether soln with  $\text{NaOH}$  (after 4 or 5 extractions). Filter the ether extractions into a weighed beaker, evaporate, dry the residue at  $100^\circ$ , and weigh. The residue should be soluble in alcohol, showing absence of most oils. If titrated with 0.1  $N$   $\text{NaOH}$ , the alcoholic soln should be practically neutral, showing absence of acid extractives, like fatty acids and salicylic or benzoic acid.

#### PHENOLPHTHALEIN IN CHOCOLATE PREPARATIONS<sup>24</sup>—TENTATIVE

78

#### REAGENTS

(a) *Iodine soln.*—Dissolve 20 g of  $\text{KI}$  in a minimum quantity of  $\text{H}_2\text{O}$ , add 14 g

of I, and when dissolved dilute to 120 cc. Add sufficient KOH soln (1+1) to discharge the I color.

(b) *Sodium sulfite soln.*—Dissolve 15 g of  $\text{Na}_2\text{SO}_3$  in  $\text{H}_2\text{O}$  and dilute to 100 cc.

79

## PREPARATION OF ALCOHOLIC EXTRACT

To a 1 g sample in a 50 cc volumetric flask, add about 35 cc of 95% alcohol; boil gently for about 20 min., rotating the flask occasionally, cool, and make up to volume with alcohol. Mix thoroly, filter thru dry paper, covering the funnel with a watch-glass to avoid evaporation, pipet a number of aliquots of 10 cc each into 250 cc beakers, and evaporate to dryness on the steam bath.

80

## DETERMINATION

Take up the residue in alkali by moistening with about 1 cc of KOH soln (1+1) and add a little  $\text{H}_2\text{O}$ . When the residue is completely in soln add a piece of ice (about 40 g), 4–4.5 cc of the prepared I reagent, and HCl from a buret, dropwise, using a stirring rod (beaker is not rotated) to complete precipitation. Then make alkaline by adding dropwise the strong KOH soln from a buret until soln is effected (except for the small quantity of fatty material that remains undissolved). Repeat the process 3 or 4 times. Then add 1 or 2 cc of the  $\text{Na}_2\text{SO}_3$  soln to the alkaline soln and filter the ice-cold mixture thru a Gooch crucible into a tall 250 cc beaker, using a bell jar arrangement and washing several times with distilled  $\text{H}_2\text{O}$ . Acidify the filtrate with HCl, using a few cc in excess, and heat on the steam bath for 20–30 min. Collect the coagulated precipitate in a weighed Gooch crucible, wash a few times with  $\text{H}_2\text{O}$ , and when sucked fairly dry wash several times with petroleum ether. Dry the precipitate in the oven (120–140°) to constant weight. Weight of precipitate  $\times 0.3872$  = weight of phenolphthalein.

## SALICYLIC ACID IN THE PRESENCE OF OTHER PHENOLS—TENTATIVE

81

## PREPARATION OF SAMPLE

*Powders.*—Weigh into a volumetric flask such a quantity of the material that an aliquot of 25–50 cc will contain approximately 0.13 g of phenol. If acid, make alkaline with 4% NaOH, adding 25 cc in excess, fill to mark with  $\text{H}_2\text{O}$ , and shake well.

*Liquids.*—Proceed as directed under 82.

82

## DETERMINATION

Transfer to a separator a sufficient quantity of soln to represent about 0.13 g of phenol. Acidify with 10%  $\text{H}_2\text{SO}_4$  and extract with ether, using 20, 15, 15 and 10 cc portions, respectively, until extraction is completed. Combine the ether in a second separator. Shake with a saturated  $\text{NaHCO}_3$  soln, using 15, 15 and 10 cc portions, and finally shake with 15 cc of  $\text{H}_2\text{O}$ . Combine the  $\text{NaHCO}_3$  soln and the washing and extract the soln with 15 cc of ether. Add the latter to the main bulk of ether and reserve for the phenol determination. Acidify the  $\text{NaHCO}_3$  soln with HCl. Extract with  $\text{CHCl}_3$ -ether solvent (2+1), using 30, 25, 20 and 10 cc, respectively, until the salicylic acid is completely removed. Filter the solvent into a beaker thru cotton previously saturated with  $\text{CHCl}_3$ . Evaporate to 5 cc on a covered steam bath with the aid of an electric fan, allowing the last 5 cc to evaporate spontaneously. Dissolve the residue in 10 cc of neutral alcohol and titrate with

0.1 *N* NaOH, using phenolphthalein as indicator. 1 cc of 0.1 *N* NaOH = 0.01381 g of salicylic acid,  $C_6H_4(OH)COOH$ .

83

MENTHOL<sup>14</sup>—OFFICIAL, FIRST ACTION

Weigh 5 g of menthol in an acetylation flask of 100 cc capacity, and add 10 cc of acetic anhydride and 1 g of powdered anhydrous Na acetate. Boil the mixture gently for 1 hour, cool, and disconnect the flask from the condenser, transferring the mixture to a small separator. Rinse the acetylation flask with 3 successive 5 cc portions of warm distilled  $H_2O$  and add the rinsings to the separator. When the liquids have completely separated, remove the aqueous layer, and wash the remaining oil with successive portions of  $Na_2CO_3$  soln (12.5 g in 100 cc of  $H_2O$ ), diluted with an equal volume of distilled  $H_2O$ , until the mixture is alkaline to 2 drops of phenolphthalein soln. Dry the resulting oil with fused  $CaCl_2$  and filter. Transfer 4–5 cc of the dry acetylated oil to a tared 100 cc Erlenmeyer flask, note the exact weight, add 50 cc of 0.5 *N* alcoholic KOH, connect the flask with a reflux condenser, and boil the mixture on a water bath for 1 hour. Allow the mixture to cool, disconnect the flask from the condenser, and titrate the excess of alkali with 0.5 *N*  $H_2SO_4$ , using 10 drops of the phenolphthalein soln as indicator. Calculate the percentage of menthol by the following formula:

$$\text{Percentage of total menthol} = \frac{A \times 7.808}{B - (A \times 0.021)}.$$

*A* is the result obtained by subtracting the number of cc of 0.5 *N*  $H_2SO_4$  required in the above titration from the number of cc of 0.5 *N* alcoholic KOH originally taken; *B* is the weight of acetylated oil taken.

THYMOL<sup>14</sup>—OFFICIAL, FIRST ACTION

84

## Quantitative Method

Weigh 2 g of pulverized thymol, transfer to a 500 cc volumetric flask, and add 25 cc of 25% NaOH soln. Agitate until the thymol is dissolved and dilute to mark at 20° with  $H_2O$ .

*Method I:* Transfer a 25 cc aliquot of the thymol soln to a 250 cc glass-stoppered Erlenmeyer flask, add 20 cc of hot HCl (1+1), and immediately run in 1–3 cc less than the theoretical amount of 0.1 *N* Br soln, 26(c). Warm to 70–80°, add 2 drops of methyl orange soln (0.1 g in 100 cc of  $H_2O$ ) and titrate slowly with the Br soln, swirling vigorously after each addition. When the red color of the methyl orange is bleached, add 2 drops of the titrating soln, stopper, shake vigorously for 10 seconds, add a drop of the methyl orange soln, and again shake vigorously for 10 seconds. Continue the addition of 2 drops of the Br soln, shaking until the red color disappears. Then add 1 drop of the methyl orange soln, shake vigorously, and if the red color does not disappear, repeat the alternate addition of 2 drops of Br soln and 1 drop of methyl orange soln, shaking after each addition as directed above, until the red color disappears. Calculate the number of cc of Br soln used to percentage of thymol. 1 cc of 0.1 *N* Br soln = 0.003753 g of thymol. Reserve the mixture in the titrating flask for *Method II*.

*Method II:* To the cooled mixture resulting from the titration according to Method I, add 3–5 cc additional Br soln. Stopper, shake, add 1 g of solid KI, wash sides of flask and stopper with distilled  $H_2O$ , and titrate the I liberated by the excess Br soln with 0.1 *N* thiosulfate soln, using starch soln, VI, 3(e), as indicator.



Calculate the amount of thiosulfate used in terms of Br soln, deduct from the total amount of Br soln added, and calculate to percentage of thymol.

To determine the approximate number of cc of Br soln required for *Method I*, heat a mixture of a 25 cc aliquot of the sample and 20 cc of HCl (1+1) to about 80° and titrate slowly with the Br soln, swirling vigorously while titrating until a yellow color, permanent for 1 min., appears.

## 85

THYMOL IN ANTISEPTICS<sup>a</sup>—TENTATIVE

If the alcoholic content is not known, make a preliminary determination of alcohol.

Transfer 50 cc (or an aliquot containing 0.05–0.10 g of thymol) to a Pt or porcelain evaporating dish. Add 6–7 cc of 50% NaOH soln, mix well, and carefully de-alcoholize by placing the dish on the steam bath before an electric fan. Evaporate a volume slightly more than the quantity of alcohol present. (If over 30% of alcohol is present, dilute with H<sub>2</sub>O to an alcoholic content of 25%. In no case should the evaporation be carried beyond 70% of the original volume.) Transfer to a 125 cc separator, washing out the evaporating dish with sufficient H<sub>2</sub>O to bring the volume to about 75 cc.

Extract the alkaline soln twice with petroleum ether, using 20 cc each time. Wash the other extracts once with 5–10 cc of 5% NaOH soln and add the washings to the aqueous layer. Extract the aqueous alkaline soln containing the thymol, together with sodium salts of boric, benzoic, and salicylic acids, with ethyl ether, making 5 extractions (20, 15, 15, 10, 10 cc). Use 8 to 10 extractions if the preparation contains glycerol.

Combine the ether extracts, transfer to a 250 cc glass-stoppered Erlenmeyer flask, add 5 cc of recently prepared alcoholic KOH soln, **XXXI**, **22**, and evaporate most of the ether, using the steam bath and an electric fan. Do not evaporate entirely to dryness but leave 6–8 cc residue. To this residue add 75 cc of hot H<sub>2</sub>O (80–90°) and 10 cc of HCl.

Immediately run in 1–3 cc less than the theoretical quantity of 0.1 N bromide-bromate soln, **26(c)**, swirling the contents of the flask constantly. Now add 2 drops of methyl orange soln and titrate slowly with the bromide-bromate soln, shaking vigorously after each addition. When the red color of the methyl orange is bleached, add 2 drops of the titrating soln, stopper, shake vigorously for 10 seconds, add one drop of methyl orange soln, and again shake vigorously for 10 seconds. Continue the addition of bromide-bromate soln, 2 drops at a time, and shake after each addition until the red color disappears. Then add 1 drop of methyl orange soln, shake vigorously, and if the red color does not disappear, repeat the alternate addition of 2 drops of the bromide-bromate soln and 1 drop of methyl orange soln, shaking after each addition, as directed above, until the red color disappears. 1 cc of 0.1 N bromide-bromate = 0.003753 g of thymol.

Test for complete extraction by shaking out the aqueous layer twice with 15–20 cc of ether and titrating the thymol, if any, in the ether extracts. Add this titration to that obtained for the main ether extract.

If the theoretical amount of thymol present is not known, add 2 drops of methyl orange soln, and titrate slowly, swirling constantly during the addition of bromine soln until the red color is bleached. Then continue according to the method outlined, beginning at the phrase: "Add 2 drops of the titrating soln, stopper, and shake vigorously . . ."

*Caution: Both the evaporation of alcohol and the later evaporation of ether must be done very carefully, in order to avoid loss of thymol by volatilization.*

#### PYRAMIDON (AMINOPYRINE)

86

##### *Qualitative Tests<sup>41</sup>—Official*

(a) Dissolve 0.01 g of the sample in 2 cc of  $H_2O$  and add a few drops of yellow  $HNO_3$  (containing nitrous acid). A purplish blue colored soln is produced.

(b) Dissolve 0.01 g of the sample in 2 cc of  $H_2O$  and add 1 cc of a 10%  $FeCl_3$  soln. A purple to violet color develops, but it becomes red on the addition of  $H_2SO_4$  (1+9).

(c) Dissolve 0.1 g of the sample in 2 cc of  $H_2O$  and add a few drops of a 5%  $AgNO_3$  soln. After a few seconds a purple to violet color is produced and on standing a deposit of metallic Ag results (useful for detecting pyramidon in antipyrin).

(d) Dissolve 0.1–0.2 g of the sample in 2 cc of  $H_2O$ , add 1 or 2 drops of a 0.2% soln of  $NaNO_2$  and a few drops of  $H_2SO_4$  (1+9), and shake for a few seconds. A purplish blue color develops, then gradually disappears, leaving a colorless soln. A large excess of  $NaNO_2$  should be avoided as it destroys the color (useful for detecting antipyrin in presence of pyramidon). On addition of a few more drops of the  $NaNO_2$  soln and the dilute  $H_2SO_4$  a yellowish green colored soln remains after the disappearance of the purple coloration if antipyrin is present.

87

##### *Quantitative Method<sup>42</sup>—Official*

Pulverize the material in a mortar and mix the powder thoroly. Place 1 g of the sample in a 100 cc volumetric flask, add 60 cc of  $N$   $HCl$ , and shake for several min. to insure complete soln of the pyramidon. Make up to the mark with  $N$   $HCl$ . Filter, if not clear, thru a dry filter, rejecting the first part of the filtrate. Pipet a 20 cc aliquot of the soln, or filtrate, into a separator; make distinctly alkaline with either  $NH_4OH$  or with 5%  $NaOH$ ; and shake out with 20, 15, 10, 10, and 5 cc portions of  $CHCl_3$ . Combine the  $CHCl_3$  extracts in a second separator and wash with 2 cc of  $H_2O$ . Filter the  $CHCl_3$  soln into a weighed beaker thru a pledget of cotton saturated with  $CHCl_3$ . Extract the wash  $H_2O$  with 5 cc of  $CHCl_3$  and add this to the combined  $CHCl_3$  extracts. Evaporate the united  $CHCl_3$  extracts just to dryness on a water bath with the aid of an electric fan and dry the residue in an oven at the temp. of boiling  $H_2O$  for 10 min. Cool in a desiccator, and weigh as pyramidon. Identify the pyramidon by means of its melting point and qualitative tests.

#### PROCAINE

88

##### *Qualitative Tests<sup>43</sup>—Official*

(a) Dissolve 0.1 g of the sample in about 10 cc of  $H_2O$ . Add 2 cc of a 5%  $KMnO_4$  soln. Warm, if necessary. Reduction occurs with evolution of gas having the odor of acetaldehyde (distinction from cocaine, which does not readily reduce  $KMnO_4$ ).

(b) Dissolve about 0.005 g of the sample in 3 cc of  $H_2O$  and add a few drops of Mayer's reagent, 176(n). With procaine a white precipitate is formed, which dissolves after the addition of a few cc of  $H_2SO_4$  (1+49). (The precipitates with stovaine and cocaine are not readily soluble in dilute  $H_2SO_4$ .)

(c) Dissolve about 0.1 g of procaine in 2 cc of  $H_2O$ . From a buret add 25 cc of 0.1  $N$   $NaOH$ . (A white precipitate is formed which dissolves in an excess of the  $NaOH$  when heated.) Heat the soln for 25 min. on a steam bath. Upon cooling the soln, extracting with  $CHCl_3$ , and evaporating the solvent, no residue should be

obtained. (Stovaine does not readily hydrolyze, and a residue giving an alkaloidal reaction remains upon evaporation of the  $\text{CHCl}_3$ .)

#### Quantitative Methods

89

##### Method I.—Official

(This method determines as procaine any *p*-amino-benzoic acid formed from the decomposition of procaine.)

Dissolve a quantity of the sample equivalent to about 0.1 g of procaine hydrochloride in 5 cc of  $\text{H}_2\text{O}$  in a 50 cc beaker. Add 25 cc of 0.1 *N* NaOH and heat on a steam bath for 25 min. Cool, and transfer the soln to a 500 cc glass-stoppered flask. Add 50 cc of standard bromide-bromate soln, 26(c), dilute with  $\text{H}_2\text{O}$  to 250 cc, add 10 cc of HCl, and stopper the flask immediately to avoid loss of Br. Shake the flask occasionally and allow to stand for 30 min. at room temp., keeping the flask tightly stoppered. (It is necessary that a large excess of Br be present, as shown by a bright yellow color.) Add quickly 10 cc of 20% KI soln, stopper, and shake the flask. Allow to stand for 15 min., shaking occasionally. Titrate the excess I with 0.1 *N*  $\text{Na}_2\text{S}_2\text{O}_3$  soln, using starch indicator, VI, 3(e). Titrate to disappearance of the blue color (the blue color that reappears on standing should be disregarded). 1 cc of 0.1 *N* bromide-bromate soln = 0.00454 g of procaine hydrochloride ( $\text{C}_{11}\text{H}_{12}\text{O}_2\text{N}_2\text{HCl}$ ).

90

##### Method II.—Official

(Determines only undecomposed procaine.)

Weigh a quantity of the powder or the number of tablets equivalent to about 0.2 g of procaine. Dissolve in 10–15 cc of  $\text{H}_2\text{O}$ , transfer the soln to a separator, and add about 3 cc of  $\text{NH}_4\text{OH}$ . Extract the ammoniacal soln 4 or 5 times with  $\text{CHCl}_3$ , using 15 cc for the first extraction and 10 cc for the subsequent extractions. Filter into a weighed beaker and evaporate the  $\text{CHCl}_3$  by means of an electric fan, preferably at room temp., avoiding prolonged heating of the procaine base, as it appears to be slightly volatile at  $100^\circ$ . Take up the residue with a slight excess of 0.1 *N* or 0.02 *N* acid. Titrate the excess of acid with 0.02 *N* NaOH, using methyl red indicator.

1 cc of 0.1 *N*  $\text{H}_2\text{SO}_4$  = 0.02726 g of  $\text{C}_{11}\text{H}_{12}\text{O}_2\text{N}_2\text{HCl}$

1 cc of 0.02 *N*  $\text{H}_2\text{SO}_4$  = 0.00545 g of  $\text{C}_{11}\text{H}_{12}\text{O}_2\text{N}_2\text{HCl}$

$\text{C}_{11}\text{H}_{12}\text{O}_2\text{N}_2 \times 1.1544$  = procaine hydrochloride (novocaine).

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#### STRYCHNINE IN TABLETS—OFFICIAL

(Other alkaloids absent.)

Count and weigh sufficient tablets (or pills) to represent 1 grain of the alkaloidal salt and transfer to a small beaker. If the color on coated tablets interferes with the indicator in titration, wash without removing the strychnine. Add 10 cc of 5% HCl, disintegrate the tablets with a stirring rod, warm on the steam bath about 10 min., cool, and transfer to a separator with not more than 10 cc of  $\text{H}_2\text{O}$ . To remove all the strychnine, add to the beaker 2 cc of 10%  $\text{NH}_4\text{OH}$  and 25 cc of  $\text{CHCl}_3$ , rinse, and add to separator. Then rinse the beaker with the portions of  $\text{CHCl}_3$  to be used for each extraction. Extract 5 times with  $\text{CHCl}_3$ , using 25, 20, 15, 10, and 5 cc portions, or until the alkaloid is completely removed. Combine the first two extractions in a second separator, into the stem of which has been inserted a pledget of absorbent cotton wet with  $\text{CHCl}_3$ . Wash with 5 cc of  $\text{H}_2\text{O}$  containing a drop of  $\text{NH}_4\text{OH}$  (1+2).

When clear, filter the  $\text{CHCl}_3$  portion into a small beaker. Wash each successive  $\text{CHCl}_3$  extract with the same wash water and filter in a similar manner into the main portion, finally washing the outer surface of the stem of the separator with a few cc of  $\text{CHCl}_3$  and adding this also to the main portion. Evaporate on a steam bath, removing the dish from the bath as the last portions evaporate to avoid decrepitation.

Add 2-5 cc of neutral alcohol, cover the beaker, and warm on a steam bath to dissolve the residue. If necessary, add just enough additional neutral alcohol to complete the soln. Add 2 drops of methyl red indicator, and titrate with 0.02  $N$   $\text{H}_2\text{SO}_4$  to a faint pink color. If more than 2 cc of alcohol was used, evaporate the excess, cool, dilute with 50 cc of recently boiled  $\text{H}_2\text{O}$ , and continue the titration with the 0.02  $N$   $\text{H}_2\text{SO}_4$  to a faint pink color. If preferred, add an excess of 0.02  $N$   $\text{H}_2\text{SO}_4$  to the alcoholic solution of the alkaloids, evaporate the alcohol, if necessary as directed above, and titrate the excess acid with 0.02  $N$   $\text{NaOH}$ .

1 cc of 0.02  $N$   $\text{H}_2\text{SO}_4 = 0.006684$  g of  $\text{C}_{21}\text{H}_{22}\text{O}_2\text{N}_2$ , 0.008565 g of  $(\text{C}_{21}\text{H}_{22}\text{N}_2\text{O}_2)_2$ .  $\text{H}_2\text{SO}_4 \cdot 5\text{H}_2\text{O}$ , or 0.007944 g of  $\text{C}_{21}\text{H}_{22}\text{O}_2\text{N}_2 \cdot \text{HNO}_3$ .

## 92 STRYCHNINE IN LIQUID PREPARATIONS<sup>65</sup>—OFFICIAL

(Other alkaloids absent.)

Measure into an evaporating dish 50 cc of the sample, or a quantity sufficient to yield at least 0.065 g of the alkaloid, and remove the alcohol by evaporation. Transfer to a separator, add 1 cc of  $\text{NH}_4\text{OH}$ , or sufficient to render the soln alkaline, and proceed as directed under 91, beginning with "Extract 5 times with  $\text{CHCl}_3$ ."

## SEPARATION OF QUININE AND STRYCHNINE<sup>66</sup>—TENTATIVE

### 93

#### REAGENT

*Bromocresol purple soln.*—Triturate 0.100 g of bromocresol purple in an agate mortar with 9 cc of 0.02  $N$   $\text{NaOH}$ . After soln dilute with  $\text{H}_2\text{O}$  to 200 cc, and filter if necessary. The soln should be deep orange to red in color. If it is purple, the addition of not more than 0.5 cc of 0.02  $N$  acid should make it red. If it is yellow, the addition of not more than 0.5 cc of 0.02  $N$  alkali should produce the red color.

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#### TOTAL ALKALOIDS

Make 50 cc of the soln acid with citric acid, add an equal volume of  $\text{H}_2\text{O}$ , evaporate to nearly the original volume to remove excess alcohol, cool, and extract with two 15 cc portions of ether to remove oily material. Make the aqueous soln alkaline with  $\text{NH}_4\text{OH}$  and extract the mixed alkaloids in the usual way with a mixture of 2 parts of  $\text{CHCl}_3$  and 1 part of ether, using 25, 20, 15, 10, and 5 cc portions. Evaporate the  $\text{CHCl}_3$  and ether in a weighed Erlenmeyer flask or beaker to dryness on a steam bath. Add a little ether and again evaporate to dryness to remove the last traces of  $\text{CHCl}_3$ . Dry at  $100^\circ$  for 1 hour and weigh to obtain the approximate weight of mixed alkaloids.

*Strychnine.*—Dissolve the alkaloidal residue in 50 cc of 10%  $\text{H}_2\text{SO}_4$ , add 5 cc of 4%  $\text{K}_2\text{Fe}(\text{CN})_6$  dropwise from a buret, stirring well, and set aside for a few hours or overnight. Collect the resulting precipitate on a small (7 cm) filter and wash 3 times with 3 cc of 5%  $\text{H}_2\text{SO}_4$ . Reserve the filtrate for the determination of quinine. Wash the precipitate immediately into a small separator with  $\text{H}_2\text{O}$ , transferring the precipitate remaining in the flask to the separator by shaking about 3 times with 3 cc of  $\text{NH}_4\text{OH}$  and a small quantity of  $\text{CHCl}_3$ . Extract the ammoniacal soln of the pre-

precipitate with 25, 15, 10, and 5 cc portions of  $\text{CHCl}_3$ . Collect the  $\text{CHCl}_3$  solns in another separator and extract the alkaloids by shaking with 25, 10, 10, and 5 cc portions of 20%  $\text{H}_2\text{SO}_4$ ; repeat the precipitation with  $\text{K}_4\text{Fe}(\text{CN})_6$  and the other operations, as above, until the  $\text{CHCl}_3$  extracts are again obtained, reserving the filtrate for determination of quinine. Evaporate the  $\text{CHCl}_3$  carefully, adding a little alcohol toward the end to prevent spattering. Weigh the residue of strychnine after drying it for 1 hour at  $100^\circ$ . (This residue should be nearly white and free from quinine.) Check volumetrically as follows: Dissolve the residue in hot alcohol, add 0.02  $N$   $\text{H}_2\text{SO}_4$  until the soln is acid to methyl red indicator, II, 55(a), then add 2 or 3 cc in excess. Evaporate most of the alcohol, cool, and titrate back with 0.02  $N$  alkali. 1 cc of 0.02  $N$  acid = 0.006684 g of strychnine,  $\text{C}_{21}\text{H}_{22}\text{O}_2\text{N}_2$  or 0.008565 g of strychnine sulfate  $(\text{C}_{21}\text{H}_{22}\text{O}_2\text{N}_2)_2\text{H}_2\text{SO}_4 \cdot 5\text{H}_2\text{O}$ .

## 95

*Quinine*.—Combine the 2 filtrates from the precipitations with  $\text{K}_4\text{Fe}(\text{CN})_6$  in a separator, make alkaline with  $\text{NH}_4\text{OH}$ , and extract with a mixture of 2 parts of  $\text{CHCl}_3$  and 1 part of ether, using 20, 15, 15, 10, and 5 cc portions of the solvent and observing the usual precaution of washing the stem of the separator with the  $\text{CHCl}_3$ -ether mixture. Wash the combined extractions in a second separator with two 5 cc portions of  $\text{H}_2\text{O}$ , transfer to a weighed beaker, evaporate to dryness, add a few cc of ether, and again evaporate to dryness to remove the final traces of  $\text{CHCl}_3$ . Dry at  $120$ – $130^\circ$ , cool, and weigh as anhydrous quinine. Test the residue qualitatively for quinine, or if desired, check the quantity volumetrically as follows: Dissolve the residue in a little alcohol, add 7 drops of the bromocresol purple indicator, then add 0.02  $N$   $\text{H}_2\text{SO}_4$  to a yellow color, and 1 cc in excess. Evaporate the soln to a small volume, cool, allow the quinine sulfate to separate, filter thru a small pledget of cotton in the stem of a funnel, wash with small portions of  $\text{H}_2\text{O}$ , and titrate the combined filtrate and washings with 0.02  $N$  alkali. 1 cc of 0.02  $N$   $\text{H}_2\text{SO}_4$  = 0.006484 g of anhydrous quinine,  $\text{C}_{20}\text{H}_{21}\text{O}_2\text{N}_3$ ; to 0.007565 g of quinine alkaloid,  $\text{C}_{20}\text{H}_{21}\text{O}_2\text{N}_3 \cdot 3\text{H}_2\text{O}$ ; or to 0.007825 g of quinine sulfate,  $(\text{C}_{20}\text{H}_{21}\text{O}_2\text{N}_3)_2\text{H}_2\text{SO}_4 \cdot 2\text{H}_2\text{O}$ .

## COCAINE\*

## 96

*Method I.—Official, First Action*

Weigh accurately a sufficient quantity of the uniformly mixed sample to represent approximately 0.1–0.2 g of the alkaloid. Transfer to a small separator and dissolve in the minimum quantity of  $\text{H}_2\text{O}$  required for soln. Make the soln slightly alkaline with  $\text{NH}_4\text{OH}$  and extract with successive small portions of peroxide-free ether until the alkaloid is completely removed from the aqueous soln, using Mayer's reagent for the test. Combine the ether extracts, remove the greater part of the ether by evaporation on the steam bath, and allow the remainder of the ether to evaporate spontaneously at room temp. Dissolve the residue in a few cc of neutral alcohol, add 20 cc of 0.05  $N$   $\text{H}_2\text{SO}_4$ , and titrate the excess of acid with 0.02  $N$   $\text{NaOH}$ , using methyl red indicator. 1 cc of 0.05  $N$   $\text{H}_2\text{SO}_4$  consumed = 0.01698 g of cocaine hydrochloride,  $\text{C}_{17}\text{H}_{21}\text{O}_4\text{NHCl}$ .

## 97

*Method II.—Tentative*

Weigh accurately a sufficient quantity of the uniformly mixed sample to represent approximately 0.2 g of the alkaloid. Dissolve in 20 cc of cold  $\text{H}_2\text{O}$ , add 2 drops of 10%  $\text{HCl}$ , and transfer to a separator. Make alkaline to litmus with a freshly

prepared saturated soln of  $\text{NaHCO}_3$  and shake out to exhaustion with petroleum ether (four 20 cc portions are usually sufficient). Run the combined extracts thru a plug of absorbent cotton into a separator and wash the cotton with petroleum ether. Add a decided excess of 0.02  $N$   $\text{H}_2\text{SO}_4$ , accurately measured, and shake vigorously for several minutes. Separate the 2 layers and wash the petroleum ether with two 10 cc portions of  $\text{H}_2\text{O}$ , adding the washings to the acid soln. Titrate the excess of acid with 0.02  $N$  alkali, using methyl red indicator, and reserve the titrated soln for the check determination described below. 1 cc of 0.02  $N$   $\text{H}_2\text{SO}_4$  required for combination with the alkaloid = 0.006793 g of cocaine hydrochloride,  $\text{C}_{17}\text{H}_{21}\text{O}_4\text{NHCl}$ .

As a check, add 10 cc of 2.5  $N$   $\text{NaOH}$  soln to the titrated alkaloidal soln and evaporate on the steam bath to a volume of about 10 cc. Cool, transfer the soln to a separator, and acidify with 10%  $\text{HCl}$ . Extract the acid soln completely with successive portions of  $\text{CHCl}_3$ . Run the combined extracts thru a plug of absorbent cotton and wash the cotton well with  $\text{CHCl}_3$ . Allow the  $\text{CHCl}_3$  soln to evaporate spontaneously in a weighed beaker, dry the residue in a vacuum desiccator for 2 hours, and weigh. From the weight of benzoic acid found calculate its equivalent of cocaine hydrochloride. 1 g of benzoic acid = 2.782 g of cocaine hydrochloride. (If desired, the quantity of benzoic acid may be determined by titration.)

#### FLUIDEXTRACT OF IPECAC

##### *Automatic Extraction Method*<sup>48</sup>—*Tentative*

98

#### PREPARATION OF SOLUTION

Pipet 20 cc of the fluidextract into a 100 cc volumetric flask, add approximately 5 cc of  $N$   $\text{H}_2\text{SO}_4$ , and with the aid of an air blast evaporate on a steam bath to a volume of about 10 cc. Then, while rotating the flask, add about 30 cc of  $\text{H}_2\text{O}$ , cool to room temp., and make up to the mark with  $\text{H}_2\text{O}$ . Allow to stand overnight and filter thru a dry filter, rejecting the first few cc of the filtrate.

99

#### DETERMINATION

Measure 20 cc of the prepared filtrate (equivalent to 4 cc of fluidextract of ipecac) into an automatic extractor (B, Fig. 49), which has been fitted to a 200 cc Erlenmeyer flask. Add 60 cc of  $\text{H}_2\text{O}$ , 2 cc of 8%  $\text{NH}_4\text{OH}$  soln, and about 50 cc of peroxide-free ether. Shake gently to prevent the deposition of any solid matter on the bottom of the extractor and then add peroxide-free ether until about 75 cc has passed over into the flask. Heat the flask on a steam bath (not electric hot plate) and extract for 2 hours, or until the extraction is complete. Separate the ether from the aqueous layer and add it to the main concentrate in the flask. Evaporate the combined ether extract on a steam bath, add 2-3 cc of absolute alcohol, and repeat the evaporation to remove all traces of  $\text{NH}_3$ . Warm the alkaloidal residue on the steam bath with 2-3 cc of neutral alcohol to insure complete soln. Add 10 cc of 0.1  $N$   $\text{H}_2\text{SO}_4$ , and dilute with about 20 cc of recently boiled, cooled  $\text{H}_2\text{O}$ . Titrate the excess of acid with 0.02  $N$   $\text{NaOH}$ , using methyl red as indicator. 1 cc of 0.1  $N$   $\text{H}_2\text{SO}_4$  = 0.024 g of ether-soluble alkaloids of ipecac.

100

##### *Hand Extraction Method*—*Tentative*

(Sometimes more rapid than the automatic extraction method and yields results almost as high.)

Pipet 20 cc of the prepared filtrate, 98, into a separator. Add 2 cc of 8%  $\text{NH}_4\text{OH}$  soln and extract the soln with equal volumes of peroxide-free ether until extraction

is completed (at least 8 times), using Mayer's reagent, 176(n), as a test. Wash the combined ether extracts in a second separator with about 10 cc of  $H_2O$  and then wash this wash  $H_2O$  with a little peroxide-free ether, adding the ether washings to the main soln. Transfer the ether soln to an Erlenmeyer flask (a 200 cc flask is a convenient size), and evaporate the ether on a steam bath with the aid of a blast of air. Add 2-3 cc of absolute alcohol and repeat the evaporation to remove all traces of  $NH_3$ . Warm the alkaloidal residue with 2-3 cc of neutral alcohol to insure complete soln, and titrate as directed in the automatic extraction method.

## 101

VOLATILE ACIDITY OF TRAGACANTH<sup>a</sup>—TENTATIVE

The quantity of volatile (acetic) acidity developed in the acid hydrolysis of gum tragacanth (*Astragalus gummifer* Lab.) affords a valuable index of the purity of this commodity when compared with results obtained by similar treatment of so-called "Indian gum" (*Cochlospermum gossypium* D. C. and *Sterculia urens* Roxb.).

Treat 1 g of the whole or powdered sample in a 700 cc round-bottomed, long-necked flask in the cold with 100 cc of  $H_2O$  and 5 cc of  $H_3PO_4$ , for several hours, or until the gum is completely swollen. Boil gently for 2 hours under a reflux condenser. A very small quantity of cellulose substance will remain undissolved.

Tragacanth yields a practically colorless soln. Indian gum gives a pink or rose soln. This reaction may be used as a preliminary test for the detection of Indian gum.

Distil the hydrolyzed product with steam, using a scrubber (Fig. 48) to connect the distillation flask with the condenser. Continue the distillation until the distillate amounts to 600 cc, and the acid residue to about 20 cc. To avoid scorching of residue do not permit concentration of contents of distilling flask to less than 20 cc. Titrate the distillate with 0.1 N NaOH soln, using 10 drops of phenolphthalein indicator, II, 10(d). Correct the result by a blank determination and express as "volatile acidity" the number of cc of 0.1 N NaOH soln required to neutralize the volatile (acetic) acid obtained.

METHYL ALCOHOL IN THE PRESENCE OF ETHYL ALCOHOL<sup>b</sup>—TENTATIVE

(Methyl alcohol present in small amounts, 5% or less.)

## 102 PREPARATION OF STOCK SOLUTION OF METHYL ALCOHOL AND REAGENT

*Soln A.*—Adjust the strength of a soln of methyl alcohol to 25% by volume ( $\pm 0.1\%$ ).

*Soln B.*—Make up 20 cc of Soln A and 95 cc of absolute alcohol (or the equivalent of this amount in dilute alcohol) to a volume of 2 liters. Make all transfers and dilutions at 20°.

*Fuchsin-sulfurous acid.*—Dissolve 0.2 g of fuchsin in 120 cc of hot  $H_2O$  and 2 g of  $Na_2SO_3$  in 20 cc of  $H_2O$ . Mix, add 2 cc of HCl, and dilute to 200 cc.

## 103

## DETERMINATION

*Total alcohols.*—Measure at room temp. (20°) 25 cc of sample, add 90 cc of  $H_2O$ , neutralize to litmus with 5% NaOH, distil, and dilute the volume of distillate to 100 cc at the same temp. as noted when the original aliquot was measured. Determine the total alcohol (as ethyl alcohol) from the sp. gr. of the distillate in the usual way and estimate the percentage of alcohol in the original soln by means of the proper dilution factor. Test a portion of this distillate by the U. S. P. test for methyl alcohol (p. 355), taking precaution to determine that  $HCHO$ , as such, is not

present. If methyl alcohol is present, transfer 10 cc of the distillate to a separator, add 40 cc of saturated salt soln, shake with 25 cc of petroleum ether, and draw off the aqueous salt soln into a distilling flask. Wash the petroleum ether in the separator with two 10 cc portions of saturated salt soln, adding these to the portion already in the distilling flask. Distil, receiving the distillate in a 50 cc graduated flask. Calculate the quantity of ethyl alcohol to add to this distillate to make a 5% soln of total alcohol (assuming it to be all ethyl alcohol) when made up to 50 cc, add this calculated amount, and make up to a volume of 50 cc. Transfer 5 cc of this distillate to a 200 cc flask for color comparison with standards.

*Color standards.*—Transfer to 200 cc flasks a series of aliquots, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, and 5.0 cc of Soln B, adding 4.5, 4.0, 3.5, 3.0, 2.5, 2.0, 1.5, 1.0, 0.5, and 0 cc, respectively, of 5% ethyl alcohol. (These amounts of methyl alcohol represent percentages in the original unknown soln when the unknown is diluted as outlined above.)

*Methyl alcohol.*—To each of the standards and to the unknown, add 1 cc of  $H_2PO_4$  (1+1) and 2 cc of 3%  $KMnO_4$  soln and allow the mixtures to stand for 10 min. Add 1 cc of 10% oxalic acid soln and allow the mixtures to stand until they have become clear or transparent. Add 5 cc of  $H_2SO_4$  soln (1+3) and 5 cc of the freshly prepared fuchsin-sulfurous acid mixture and allow the solns to stand for 1½ hours. Dilute to 200 cc, mix thoroly, and transfer equal quantities to a series of test tubes of uniform color and diameter for color comparison. Compare the unknown with the standard which it most nearly approaches in color intensity, approximating intervals less than 0.5%, if desired. The value obtained represents the percentage of methyl alcohol in the original sample.

*Ethyl alcohol.*—Deduct the percentage of methyl alcohol, as determined colorimetrically, from the percentage of total alcohols, as previously determined.

#### CHLOROFORM IN MIXTURES<sup>91</sup>—TENTATIVE

##### 104

##### REAGENTS

(a) *Alcoholic potassium hydroxide.*—Dissolve 30 g of KOH (free from chloride) in 30 cc of  $H_2O$  and sufficient methyl alcohol to make 100 cc. Allow to stand several days and decant the clear liquid.

(b) *Silver nitrate.*—Dissolve 10 g of  $AgNO_3$  in sufficient  $H_2O$  to make 500 cc.

(c) *Phenolphthalein.*—Dissolve 1 g in sufficient 95% alcohol to make 100 cc.

##### 105

##### DETERMINATION

Place 1 g of  $CaCO_3$  and 75 cc of alcohol in a 250 cc Kjeldahl distilling flask and carefully pipet into this mixture 20 cc of the sample, being careful not to agitate the mixture and to keep the tip of the pipet just below the surface of the liquid. Connect the flask with a straight-bore condenser and distil into a previously cooled citrate bottle immersed in cracked ice and containing 25 cc of the alcoholic KOH soln into which the tip of the delivery tube should extend.

When 70 cc of the alcohol has been distilled over (judged by marking the bottle previously), discontinue the distillation and wash the receiving tube with about 10 or 15 cc of distilled  $H_2O$ , collecting the washings in the citrate bottle. Stopper the bottle; gently agitate, taking care to prevent the soln from coming in contact with the rubber washer; and allow to stand overnight at room temp. Heat on a steam bath for 1 hour, remove from the bath and allow to cool. Empty the contents of the bottle into a 500 cc beaker and wash the bottle with distilled  $H_2O$  until the washings



are no longer alkaline to phenolphthalein, adding each washing to the main soln. Now add 15 cc of  $\text{HNO}_3$  and an excess of  $\text{AgNO}_3$ , stir well, and allow the mixture to stand in a dark place for 15 min. Collect the precipitate upon a Gooch crucible that has been previously prepared, dried at  $105^\circ$ , and weighed. Wash the precipitate with several portions of distilled  $\text{H}_2\text{O}$ , then with 5 cc of alcohol followed by a 5 cc portion of ether. Dry at  $105^\circ$  and weigh.

1 g of  $\text{AgCl} = 0.27764$  g of  $\text{CHCl}_3$ . Assuming that 1 minim of  $\text{H}_2\text{O}$  weighs 0.06161 g and that the sp. gr. of  $\text{CHCl}_3$  is 1.476 (average of the U.S.P. limits), then 1 minim of  $\text{CHCl}_3$  weighs 0.090936 g, and basing calculations on 1 fl. oz. measuring 29.57 cc, the factor for grams of  $\text{AgCl}$  to minims of  $\text{CHCl}_3$  per fluid ounce is 4.5142 for the 20 cc sample.

#### CHLOROFORM AND CARBON TETRACHLORIDE<sup>24</sup>—TENTATIVE

##### 106

##### REAGENTS

(a) *Alcoholic potassium hydroxide*.—Dissolve 35 g of KOH in sufficient methyl alcohol to make 100 cc. (This is a saturated soln.)

(b) *Ammonium or potassium thiocyanate soln*.—0.05 N. Adjust by titrating against 0.1 N  $\text{AgNO}_3$  soln.

(c) *Ferric ammonium sulfate indicator*.—Dissolve 8 g of  $\text{Fe}(\text{NH}_4)(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$  in sufficient  $\text{H}_2\text{O}$  to make 100 cc.

##### 107

##### WEIGHING OF SAMPLE

###### (1) *Chloroform or Carbon Tetrachloride*:

Carefully transfer 30 cc of alcoholic KOH soln into an air-dried, 60–70 cc pressure bottle and stopper. Do not moisten the neck of the bottle with the reagent. (Use 104(a) for  $\text{CHCl}_3$  and 106(a) for  $\text{CCl}_4$ , and for mixtures of  $\text{CHCl}_3$  or  $\text{CCl}_4$ .) Weigh the stoppered bottle with the contents. (Conveniently done by suspending the bottle on the balance by means of the clamp that holds the stopper.)

Immediately after opening the bottle, add about 1 cc of the sample from a 1 cc pipet, holding the pipet just above the top level of the reagent in the pressure bottle. As the level of the reagent rises with the draining of the sample into the bottle, raise the pipet correspondingly so as to avoid contact with the reagent. Avoid having the bottle open longer than necessary, 20 seconds being a convenient time. Stopper the bottle so as to assure a tight fit and weigh. Determine the weight by difference. Proceed as directed under 108.

###### (2) *Carbon Tetrachloride in Capsules*:

Ascertain the gross weight of a representative number of capsules. Open the capsules and transfer the contents to a suitable flask. Weigh the dried empty capsules and determine the average net contents. Proceed as directed under (1), using the composite sample.

###### (3) *Chloroform or Carbon Tetrachloride Mixtures*:

Proceed as directed under (1), using not more than 10 cc of the mixture containing 0.08–1.6 g of  $\text{CHCl}_3$  or  $\text{CCl}_4$ . Note the temp. of the mixtures. Ascertain the volume-equivalent of the weighed sample. Weigh a definite volume of the mixture at the same temp., using a 50 or 100 cc volumetric flask, and calculate.

**NOTE.**—If desired, the sample may be measured directly with a pipet instead of being weighed, or a measured volume may be diluted with methyl alcohol to some definite volume and thoroly mixed and a suitable aliquot of this dilution used for the determination.

## 108

## DETERMINATION

If the sample is a mixture, mix the contents of the bottle by gentle swirling and allow the bottle to stand about an hour (30 min. is sufficient for  $\text{CHCl}_3$ , pure or nearly so). Place the bottle in a wire basket and set the basket in a water bath at room temp. Invert a tin can over the bottle and cover with a towel to prevent injury to the analyst in case the bottle should burst. Heat the bath to boiling and maintain at this temp. for 1 hour (15 min. is sufficient for  $\text{CHCl}_3$ , pure or nearly so).

Cool gradually, transfer the contents of the pressure bottle to a 200 cc volumetric flask, and wash out the bottle thoroly with  $\text{H}_2\text{O}$ , draining the washings into the flask. Bring to room temp., fill to mark with  $\text{H}_2\text{O}$ , and mix.

Transfer a suitable aliquot to a 100 cc volumetric flask and acidify with  $\text{HNO}_3$ , adding about 2 cc in excess. Add 25 or 50 cc of 0.1  $N$   $\text{AgNO}_3$  (an excess), shake thoroly, fill to mark with  $\text{H}_2\text{O}$ , and mix. Filter the mixture thru a dry filter into a dry flask, rejecting the first 20 cc of the filtrate. To a 50 cc aliquot of the filtrate, add 3 cc of the ferric ammonium sulfate indicator and titrate the excess 0.1  $N$   $\text{AgNO}_3$ , using 0.05  $N$   $\text{NH}_4$  or  $\text{K}$  thiocyanate.

If the original sample contains chloride, which is precipitated by  $\text{AgNO}_3$ , determine the quantity and make correction.

If the original sample contains sugar or other organic material and (after the saponification of the  $\text{CHCl}_3$  or  $\text{CCl}_4$  and dilution of the mixture with  $\text{H}_2\text{O}$ ) is highly colored, thus interfering with the titration, transfer the contents of the pressure bottle to a  $\text{Ni}$  crucible with the aid of  $\text{H}_2\text{O}$ . Evaporate to dryness and char the residue. Allow to cool, treat with  $\text{H}_2\text{O}$ , filter into a suitable volumetric flask, washing the residue and filter with  $\text{H}_2\text{O}$  until free from chloride. Fill to mark with  $\text{H}_2\text{O}$  and mix. Determine the chloride as directed above.

Make a blank test, using in the pressure bottle the same quantities of solvents and reagents as when the sample is present, and apply such correction as may be necessary. 1 cc of 0.1  $N$   $\text{AgNO}_3$  = 0.003979 g of  $\text{CHCl}_3$  or 0.003846 g of  $\text{CCl}_4$ .

## TETRACHLORETHYLENE IN MIXTURES—TENTATIVE

## 109

## REAGENTS

(a) *Metallic sodium*.—Place 10 cc of xylene and 2 g of metallic  $\text{Na}$  in a small Erlenmeyer flask fitted with a glass stopper, adding more xylene if necessary to cover the metal. Heat on a hot plate until the  $\text{Na}$  is melted. Shake to remove excess vapor, stopper, wrap in a towel, and shake vigorously until the  $\text{Na}$  is finely divided. Cool, remove the xylene, and replace with 5 cc of fresh xylene.

(b) *Ferric ammonium sulfate indicator*.—Dissolve 8 g of  $\text{Fe}(\text{NH}_4)(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$  in sufficient  $\text{H}_2\text{O}$  to make 100 cc.

## 110

## DETERMINATION

Weigh carefully a 125 cc cork-stoppered Erlenmeyer flask. Remove from balance pan, open, and from a split cc pipet add sufficient sample to give the equivalent of about 0.16 g of tetrachlorethylene. Stopper securely and weigh again.

To the contents of the flask add 10 cc of xylene and 2 g of the  $\text{Na}$  reagent. Connect the flask to a reflux condenser, using a cork stopper protected by tin foil, and heat on a hot plate to boiling. Add about 1 cc of amyl alcohol thru the condenser. Reflux gently for 2 hours and add at intervals 1 cc portions of amyl alcohol until a total of 5 cc is added. Disconnect the flask. When cool, destroy the excess of  $\text{Na}$  by the cautious addition of 20 cc of  $\text{H}_2\text{O}$ . After all action has subsided, acidify

with  $\text{HNO}_3$  and transfer the mixture to a separator. Wash the xylene layer with three 10 cc portions of  $\text{H}_2\text{O}$  and filter the acid, aqueous solns into a 200 cc volumetric flask. Add 50 cc of 0.1  $N$   $\text{AgNO}_3$  soln to the flask and make up to 200 cc. Shake thoroly and pour thru a dry filter, discarding the first 20 cc of filtrate. To a 100 cc aliquot add 3 cc of the indicator. Titrate the excess of  $\text{AgNO}_3$ , using the 0.05  $N$   $\text{NH}_4\text{CNS}$ . Make a blank test for chloride. 1 cc of 0.1  $N$   $\text{AgNO}_3 = 0.004146$  g of  $\text{C}_2\text{Cl}_4$ .

The chloride may also be determined gravimetrically. 1 g of  $\text{AgCl} = 0.2892$  g of  $\text{C}_2\text{Cl}_4$ .

#### BARBITAL AND PHENOBARBITAL<sup>14</sup>—OFFICIAL

(Applicable in absence of stearic acid.)

111

#### REAGENTS

(a) *Alkaline salt soln.*—Dissolve 20 g of  $\text{NaOH}$  in  $\text{H}_2\text{O}$ , dilute to 1 liter, add  $\text{NaCl}$  to saturation, and filter.

(b) *Solvent.*—Mix 20 cc of ether and 80 cc of  $\text{CHCl}_3$ .

112

#### DETERMINATION

Transfer 0.3 g of the powdered sample to a separator and dissolve in 10 cc of the alkaline salt soln. If tablet lubricants (other than stearic acid) are present, wash with 15 cc of ether and decant from top of separator into a small beaker. Repeat the extraction with ether twice. Add 2 cc of  $\text{HCl}$  to the alkaline soln, then 5 cc of  $\text{H}_2\text{O}$  to prevent precipitation of salt. Extract with  $\text{CHCl}_3$ -ether 5 times, using 30, 20, 20, 10, and 10 cc portions of the solvent. Test for complete extraction with 10 cc of solvent and evaporate in a separate beaker. Combine the solvent in a second separator and wash with 2 cc of  $\text{H}_2\text{O}$  acidified with a drop of  $\text{HCl}$ . Filter the solvent thru a pledget of cotton into a small weighed beaker. Evaporate on a steam bath with the aid of an electric fan, heat for 10 min. at  $90$ – $100^\circ$ , cool in a desiccator, and weigh.

113

#### Alternative Method<sup>14</sup>—Tentative

(Applicable in presence of stearic acid.)

Dissolve the residue obtained, 112, in 10 cc of alcohol, add 20 cc of a saturated, aqueous soln of  $\text{Ba}(\text{OH})_2$ , and stir well. Filter into a separator and wash the residue and filter with two or three 10 cc portions of the  $\text{Ba}(\text{OH})_2$  soln. Acidify the soln with 10%  $\text{HCl}$  and proceed as directed under 112, beginning with the words, "Extract with  $\text{CHCl}_3$ -ether 5 times."

114

#### ACONITINE IN ACONITE ROOT<sup>14</sup>—TENTATIVE

Crush and macerate the aconite root in a mortar with 15 cc of  $\text{H}_2\text{O}$  and transfer to the extraction tube (Mojonnier type). Add 5 cc of 10%  $\text{NH}_4\text{OH}$  and extract 2 or 3 times with 15 cc portions of ether. Transfer the ethereal extract to a separator and wash with  $\text{H}_2\text{O}$ . Extract the washed, ethereal extract with 2 or 3 cc of 0.02  $N$   $\text{H}_2\text{SO}_4$ . Test the aqueous layer with methyl red indicator; if alkaline, discard the aqueous layer. Continue to extract with 2 or 3 cc of 0.02  $N$   $\text{H}_2\text{SO}_4$ , until the aqueous layer remains acid to methyl red indicator. Test the slightly acid aqueous layer for aconitine by the following method:

In a small test tube add 1 or 2 drops of 5%  $\text{Na}_2\text{CO}_3$  to 1 or 2 cc of the slightly acid, aqueous soln. Heat to  $60^\circ$ , stirring with a thermometer. Cool, and transfer a

few drops of the liquid to a micro-slide and examine the crystals. Irregular hexagonal plates are formed by aconitine. Most characteristic crystals of aconitine are formed in solns of a strength of 1:1000 or less.

#### CASCARA SAGRADA<sup>17</sup>—TENTATIVE

115

##### REAGENT

*Sodium bicarbonate soln.*—(5+100). Make up in cold H<sub>2</sub>O as needed; add 1 cc of 0.1 N HCl to insure freedom from carbonates.

116

##### DETERMINATION

Introduce CHCl<sub>3</sub> into the continuous extraction apparatus (A, Fig. 49) to within 5 cm of the overflow. Adjust a 200 cc Erlenmeyer flask carrying 125 cc of CHCl<sub>3</sub> to the apparatus with a well-fitted, tin-foiled cork. Into the inner tube of the apparatus introduce a measured or weighed portion of the sample representing approximately 2 g of cascara sagrada. Add 20 cc of H<sub>2</sub>O and 1 cc of acetic acid (1+100) to the cascara layer. Connect the apparatus to the condenser. (The outlet of the condenser should not be constricted. If it is, place a hole in the side near its tip to insure free return of CHCl<sub>3</sub>.)

Adjust the burner, using an asbestos ring to prevent overheating, and reflux rapidly for 2 hours. (The CHCl<sub>3</sub> in the tube will be colorless.) Disconnect the flask and discard its contents.

Recharge the Erlenmeyer flask with 125 cc of CHCl<sub>3</sub> and connect to the apparatus, which still carries the CHCl<sub>3</sub>-exhausted acetic acid soln of the original sample and the clear exhausted CHCl<sub>3</sub>. Add 10 cc of H<sub>2</sub>SO<sub>4</sub> (1+1) to the cascara layer by means of a pipet.

Connect the apparatus to the condenser, adjust the burner, and reflux rapidly. At the end of 3 hours, the CHCl<sub>3</sub> in the apparatus should be practically colorless, but it may contain a small amount of color, a non-emodin material.

Remove the flame and disconnect the flask. Transfer the CHCl<sub>3</sub> in the flask to a separator, wash the flask with 10 cc of H<sub>2</sub>O, and transfer the H<sub>2</sub>O to the separator carrying CHCl<sub>3</sub>. Shake, withdraw the CHCl<sub>3</sub>, and again wash the H<sub>2</sub>O with 10 cc of CHCl<sub>3</sub>, adding the washings to the main CHCl<sub>3</sub> soln. Wash the CHCl<sub>3</sub> with three 10 cc portions of the sodium bicarbonate soln, then wash the combined reagent with CHCl<sub>3</sub> two or three times. Discard the aqueous soln.

Shake out the combined CHCl<sub>3</sub> to exhaustion with saturated sodium carbonate soln in a train of separators. (Four 10 cc portions should suffice.) Wash the combined reagent with CHCl<sub>3</sub> several times. Discard all the CHCl<sub>3</sub>.

Add sufficient HCl (1+1) to the aqueous soln (cautiously, a few cc's at a time) to insure an acid reaction. Extract with CHCl<sub>3</sub> in a separator or automatic extractor to completion. Combine the CHCl<sub>3</sub> and wash with 5 cc of H<sub>2</sub>O. Filter the CHCl<sub>3</sub> thru a filter wetted with CHCl<sub>3</sub>. Evaporate to 20 cc. Transfer the residue to a small glass or Pt dish, evaporate to dryness, and dry at 100° for 2 hours. Cool, and weigh the hydrolyzed products from the anthraglucosides of cascara.

#### ALKALOIDS IN ERGOT<sup>18</sup>—TENTATIVE

(Applicable to ergotamine and ergotoxine.)

117

##### REAGENTS

(a) *Dimethylaminobenzaldehyde.*—Add 650 cc of H<sub>2</sub>SO<sub>4</sub> to about 300 cc of H<sub>2</sub>O. Cool, add 1.25 g of paradimethylaminobenzaldehyde, 0.05 g of ferric chloride, and sufficient H<sub>2</sub>O to make 1 liter.

(b) *Ergotoxine ethanesulfonate standard soln.*—With the aid of an excess of tartaric acid, dissolve sufficient ergotoxine ethanesulfonate, accurately weighed, gradually adding  $H_2O$ , to yield a soln containing 0.01 g of ergotoxine ethanesulfonate and 0.05 g of tartaric acid in 100 cc.

(c) *Ergotamine tartrate standard soln.*—Prepare as directed in (b), using ergotamine tartrate instead of ergotoxine ethanesulfonate.

## 118

## EXTRACTION OF ALKALOIDS

Pipet 5 cc of the fluidextract at  $20^\circ$  into a separator and dilute with 30 cc of  $H_2O$ . Add 2 cc of  $NH_4OH$  (1+10) or until distinctly alkaline to litmus paper. Extract with U.S.P. peroxide-free ether, using 40, 25, 20, 15 cc portions, or until the alkaloids are removed completely. To assure complete extraction make an additional extraction with 20 cc of ether, evaporate in a separate beaker, dissolve the residue in 1 cc of 1% tartaric acid soln, and test with reagent for blue color. Combine the ether extractions in a separator, wash at least 3 times with 25 cc portions of  $H_2O$  containing three drops of  $NH_4OH$  to remove the yellow pigments, and finally wash twice with  $H_2O$  to remove the excess of alkali. Transfer the combined washings to a separator, extract with three 5 cc portions of ether, wash the combined portions with  $H_2O$ , and add to the main ether extractions. Shake the ether with an aqueous 1% tartaric acid soln, using 10, 10, 10, and 5 cc portions, respectively, until the alkaloids are removed completely. Evaporate the combined acid solns on a water bath in a current of air to remove the ether, transfer the soln to a 25 cc volumetric flask, and make to volume.

## 119

## COLORIMETRIC COMPARISONS

Pipet 1 cc of the standard soln at  $20^\circ$  to a glass colorimeter cup or test tube and add 2 cc of the reagent. Mix. Pipet 1 cc of the extracted ergot soln to a second cup or test tube and add 2 cc of the reagent. Mix. Allow to stand for 30 min., or until the blue color reaches maximum intensity. Read in a colorimeter. Repeat the comparison if necessary with aliquots of the alkaloidal solns to produce about the same color intensity as the standard. Calculate the percentage of total alkaloids of ergot as ergotamine tartrate or ergotoxine ethanesulfonate.

ETHER<sup>99</sup>—TENTATIVE

(Not applicable in the presence of essential oils.)

## 120

## REAGENTS

(a) *Sulfuric acid.*—(1+1). Carefully add  $H_2SO_4$  to an equal volume of  $H_2O$  and cool to room temp.

(b) *Potassium dichromate soln.*—1 N. Dissolve 49.035 g of pure  $K_2Cr_2O_7$  (or corresponding quantity of known purity) in sufficient  $H_2O$  to make 1 liter.

(c) *Sulfuric acid-potassium dichromate soln.*—0.5 N. Carefully add 500 cc of  $H_2SO_4$  to 500 cc of 1 N  $K_2Cr_2O_7$  soln (accurately measured in a volumetric flask), and cool to room temp. Use two 1 liter flasks for mixing and cooling. Transfer to a 1 liter volumetric flask, add the  $H_2SO_4$  for washing, and fill to mark with the  $H_2SO_4$ . Mix thoroly.

Standardize against 0.05 N Na thiosulfate soln as follows:

Pipet exactly 25 cc of Reagent (c) into a 250 cc ground-glass stoppered volumetric flask and dilute to mark with  $H_2O$  after cooling to room temp. Mix thoroly. Pipet a 50 cc aliquot into a 500 cc ground-glass stoppered flask; add 100 cc of  $H_2O$ ,

10 cc of  $\text{H}_2\text{SO}_4$ , and 10 cc of 25% KI soln, freshly prepared. Stopper flask and allow to stand for 3–5 min. Add 150–200 cc of  $\text{H}_2\text{O}$  and titrate with 0.05 *N* Na thiosulfate, using starch soln, VI, 3(e), freshly prepared, as indicator.

## 121

## APPARATUS

Set up the apparatus as illustrated, Fig. 50. Beginning at the air intake end of the aspiration train, use a 400 cc bottle as wash bottle (A), six 50 cc graduated cylinders, having an inside diameter of 1.5 cm and a height of 32–35 cm (B–C–D–E–F–G), a 500 cc bottle as safety reservoir (H), and a 400 cc bottle as wash bottle (I), which is supplied with a soda-lime tube. Supply each container with a closely fitting rubber stopper and vapor carrying tubes. The intake tube should extend almost to the bottom, and the outlet tube, 1 cm. below the rubber stopper. Use heavy-walled glass tubing having an outside diameter of 5 mm. Draw the outlets of all vapor carrying tubes down to small openings. Use heavy-walled rubber tubing for connections and between cylinders expose only 0.5–1 cm to the vapors.

## 122

## PREPARATION OF SAMPLE

Carefully weigh a 100 cc glass-stoppered volumetric flask containing 65–70 cc of  $\text{H}_2\text{O}$ . Pipet 5 cc of ether, holding the pipet just above the  $\text{H}_2\text{O}$  in the flask, and as the level of the  $\text{H}_2\text{O}$  is raised by the draining of the ether into the flask, raise the pipet correspondingly to avoid contact with the  $\text{H}_2\text{O}$ . Immediately stopper the flask and weigh. The difference in weight is the weight of ether. Carefully and gently swirl the liquid in the flask until the ether is dissolved and then fill to mark with  $\text{H}_2\text{O}$ . Stopper flask and thoroly mix.

If the unknown ether sample is an alcoholic or hydroalcoholic soln, prepare a soln by dilution with  $\text{H}_2\text{O}$  to meet the requirements given under 124.

## 123

## PRELIMINARY CHARGING OF APPARATUS

Transfer about 100 cc of the 0.5 *N* sulfuric acid-potassium dichromate soln to wash bottle A, and 35 cc of the  $\text{H}_2\text{SO}_4$  soln to each cylinder, C and D. (Use a funnel with a long stem to avoid wetting upper portion of container.) Pipet 40 cc, 25 cc, 25 cc of 0.5 *N* sulfuric acid-potassium dichromate soln, into cylinders E, F and G, respectively, avoiding unnecessary wetting of the outside of the stem of the pipet and touching the inside of cylinder with the wetted stem of the pipet while draining. Bottle H remains empty. Transfer about 50 cc of  $\text{H}_2\text{SO}_4$  to bottle I and fill tube J with an appropriate quantity of soda-lime, layered on bottom and top with cotton. Stopper tightly all containers except cylinder B. Leave all rubber tubing connections between cylinders and glass stopcocks K and L open.

## 124

## DETERMINATION

If the sample is known not to contain alcohol or other substances which will be oxidized by the sulfuric acid-potassium dichromate soln, pipet an aliquot as directed above into a 250 cc ground-glass stoppered flask containing 50 cc of the same reagent. Stopper flask, swirl gently, and allow to stand for 1 hour. Titrate the excess acid dichromate and calculate as directed below.

Pipet an aliquot of the sample containing 0.035–0.2 g of ether in aqueous soln or hydro-alcoholic soln, containing not more than 5 g of alcohol, into cylinder B containing sufficient  $\text{H}_2\text{O}$  to make a total volume of 25 cc. Hold the pipet just above the top level of the liquid in the cylinder, and as the liquid is raised by the draining of the sample, raise the pipet correspondingly so as to avoid contact with the liquid.

Stopper tightly and immediately connect with cylinder C and wash bottle A. Connect the suction pump at M, and with stopcock L about half open start the pump. With bottle H and cylinder G connected, gradually close stopcock K until a slow current of bubbles passes thru the reagent in cylinder F and connect cylinder E. Repeat until cylinder B, which contains the sample, is connected. Make certain all connections are air-tight. (Usually stopcock L requires no further adjustment.) Carefully adjust stopcock K until a rapid and steady current of bubbles (about 150 per min.) flows thru the aspiration train. (Usually this is attained with cock K slightly open, depending upon the size of the opening thru cock L.) Take care not to have any of the reagent touch the rubber stopper by spray or otherwise. As they rise in cylinders B and C the bubbles increase in size, couple up, and near the surface each bubble occupies the entire cross-section of the cylinder and has a vertical height of 1-1.5 cm.

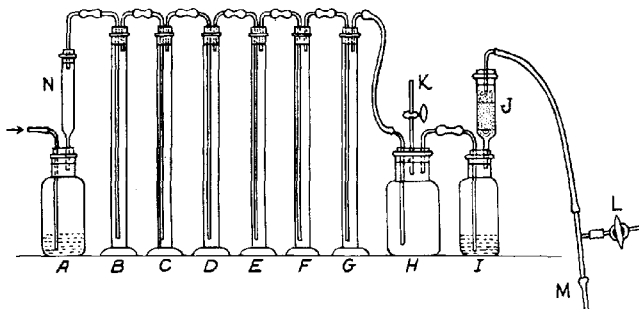


FIG. 50. --APPARATUS FOR THE DETERMINATION OF ETHER

Aspirate for 5 hours. If not certain that all the ether has been carried over into the 0.5 *N* acid-dichromate soln, discontinue the aspiration as directed in the following paragraph. Transfer the contents of cylinder *E* to a ground-glass stoppered 500 cc volumetric flask. Pipet 25 cc of the acid dichromate soln into cylinder *E*. Aspirate as before.

Gradually open cock *K* until the rate of flow of the bubbles is appreciably slower and disconnect the rubber tubing between cylinders *B* and *C*. Then gradually open cock *K* as before and disconnect the tubing between *C* and *D*. Repeat until all cylinders are disconnected.

Transfer the acid-dichromate soln (contents of cylinders *E*, *F* and *G*) to a ground-glass stoppered 500 cc volumetric flask. Wash the cylinders and glass tubes with  $H_2O$  and drain washings into the flask. Add 200-300 cc of  $H_2O$  and cool. Add more  $H_2O$  and again cool to room temp. Make up to volume and mix thoroly.

Pipet a 25 cc aliquot into a 500 cc ground-glass stoppered flask and continue as directed under (c), beginning with "add 100 cc of  $H_2O$ ."

Calculate the 0.5 *N* sulfuric acid-potassium dichromate soln consumed by the sample. 1 cc of 0.5 *N* acid-dichromate = 0.00463 g of ether.

#### 125

#### MORPHINE IN SIRUPS—TENTATIVE

Shake the bottle well and transfer 50 cc to a 150 cc pear-shaped separator. Add a few drops of 10% ammonia water to insure a weak alkaline reaction and test with

litmus paper. Extract the total alkaloids with a mixture of  $\text{CHCl}_3$  and alcohol (9+1). (About seven 25 cc portions are necessary, depending on care of separating the solvent and length and violence of each shake-out. Larger amounts of solvent may be used to insure absence of emulsions. Combine the solvents and wash with 5 cc of  $\text{H}_2\text{O}$ . Run thru chloroform-wetted cotton. Evaporate the solvent. (If the sirup is known to carry pure morphine alkaloid or its salt, and no other alkaloid, this residue may be dissolved in alcohol and filtered, and the soln titrated.) Dissolve in 2 cc of 5%  $\text{HCl}$  on a water bath, covering the beaker to insure complete soln. Add 20 cc of  $\text{H}_2\text{O}$  and transfer to a separator. Make alkaline with 5 cc of 5%  $\text{KOH}$  soln and exhaust with three 20 cc portions of  $\text{CHCl}_3$  followed by two 20 cc portions of petroleum ether (removal of non-phenolic alkaloids and  $\text{CHCl}_3$ ). Combine immiscible solvents and wash with 5 cc of  $\text{H}_2\text{O}$ . Discard the solvent and add the wash water to main, aqueous soln.

Render the aqueous soln acid with 5%  $\text{HCl}$  and then just alkaline with a few drops of 10% ammonia and extract with three 20 cc portions of petroleum ether for removal of petroleum ether-soluble phenolic alkaloids. Wash the combined petroleum ether with 5 cc of  $\text{H}_2\text{O}$ . Discard the petroleum ether. Add the wash water to the main, aqueous soln. Saturate with salt.

Extract the morphine completely with seven 25 cc portions of the  $\text{CHCl}_3$ -alcohol mixture. Combine the solvent. Wash with 5 cc of  $\text{H}_2\text{O}$  and run solvent thru a plug of  $\text{CHCl}_3$ -saturated cotton. Evaporate the solvent. Dissolve the residue in 5 cc of neutralized alcohol in a covered beaker by aid of heat on the steam bath. Add an excess of 0.02  $N$   $\text{H}_2\text{SO}_4$  and titrate back with 0.02  $N$  alkali, using methyl red indicator. 1 cc of 0.02  $N$   $\text{H}_2\text{SO}_4$  = 0.0076 g of morphine sulfate,  $(\text{C}_{17}\text{H}_{19}\text{NO}_3)_2 \cdot \text{H}_2\text{SO}_4 \cdot 5\text{H}_2\text{O}$ .

#### SWELLING FACTOR OF PSYLLIUM<sup>61</sup>—TENTATIVE

126

##### APPARATUS

Select the required number of 50 cc graduated cylinders and provide them with one-holed rubber stoppers (usually Nos. 3 or 4). Thru the stoppers insert glass stirring rods of such diameters that they will slide easily in the holes and of such lengths that any material in the bottom of the cylinders may be stirred conveniently and thoroly.

127

##### DETERMINATION

Place 1 g of psyllium (seeds) in a cylinder, add  $\text{H}_2\text{O}$  to the 20 cc mark, and stir well. Draw the rod out of the liquid after each stirring by sliding thru the stopper. Place the cylinder and its contents in a refrigerator or a cool place ( $5$ – $10^\circ$ ) for 24 hours, stirring the contents at frequent intervals. Remove the cylinder, stir, and allow the contents to settle for 1 hour at room temp. (or until no further change is observed in the total volume occupied by the drug). This final reading (to one decimal place) is taken as the swelling factor of the drug. (Note: In the test on *Lallemantia Royleana*, add  $\text{H}_2\text{O}$  to the 50 cc mark in the cylinder.)

#### SANTONIN IN MIXTURES AND TABLETS<sup>62</sup>—TENTATIVE

128

##### Langer's Method (Modified)

Weigh out a sample equivalent to approximately 0.15 g of santonin, and extract with 10, 10, 10, 5 and 5 cc portions of petroleum ether saturated with santonin. (If the sample is fat-free this step may be omitted.) Filter each portion of solvent with the aid of suction to complete dryness thru a Gooch crucible provided with an asbestos mat before following with another portion of fresh solvent. Extract the



residue in the solution flask and the crucible with 15, 10, 5 and 5 cc of hot benzol, filtering each portion as before. Evaporate the benzol extract in a tared flask and dry the residue to constant weight at 100°. Weight of the santonin in the flask equals the weight of santonin in the sample.

#### SANTONIN IN MIXTURES<sup>129</sup>—TENTATIVE

129

##### REAGENT

*Dinitrophenylhydrazine sulfate soln.*—Dissolve 1 g of 2:4 dinitrophenylhydrazine in a mixture of 90 cc of H<sub>2</sub>O and 10 cc of H<sub>2</sub>SO<sub>4</sub> by warming, cool, and filter.

130

##### DETERMINATION

Weigh 2.5 g of the ground sample into a Gooch crucible and wash with about 100 cc of petroleum ether saturated with santonin. Discard the washings. Extract with about 100 cc of hot benzol, collecting the filtrate in a beaker. Evaporate to dryness, warm the residue with alcohol until dissolved, transfer to a 100 cc volumetric flask, cool, make to volume at 20° with alcohol, and filter if necessary. To 25 cc of the soln add 50 cc of the dinitrophenylhydrazine soln and allow to stand for 48 hours in a dark place. Collect the precipitate in a Gooch crucible and wash it with dilute alcohol (1+2), using a total volume of about 150 cc. Dry the residue for 1 hour at 100°, cool, and weigh. Weight of precipitate  $\times 0.5775$  = weight of santonin.

131

#### SANTONIN IN SANTONICA (LEVANT WORM SEED)<sup>131</sup>—TENTATIVE

Extract 3 g of the ground sample with benzol in a Soxhlet apparatus or an automatic percolator (Fig. 51), for 3 hours. Wash the extract into a separator with a little benzol, add more benzol if necessary to make a total volume of approximately 100 cc, and shake vigorously for 5 min. with 35 cc of 8% Na<sub>2</sub>CO<sub>3</sub> soln. Allow the mixture to separate completely and transfer the aqueous layer to a second separator. Wash the benzol once with 10 cc of H<sub>2</sub>O and add the washing to the second separator. Shake the combined aqueous extracts with 10 cc of benzene, discard the aqueous layer, wash the benzol with 5 cc of H<sub>2</sub>O, and combine with the benzol in the first separator. Filter the benzol soln thru cotton and evaporate the filtrate to dryness. Warm the residue with 5 cc of alcohol until the mass is disintegrated, and add 60 cc of saturated aqueous soln of Ba(OH)<sub>2</sub> while stirring. Heat the mixture to boiling, place on the steam bath for 10 min., filter into a separator, and wash the filter and beaker with two 10 cc portions of hot Ba(OH)<sub>2</sub> soln. Add 6 cc of HCl (2+1) to the filtrate, cool, and extract with 25, 15, 10, 10, and 5 cc portions of CHCl<sub>3</sub>, filtering thru a pledget of cotton in the stem of the funnel, and evaporate the filtrate to dryness. Dissolve the residue in 25 cc of alcohol by warming, mix the soln with 50 cc of dinitrophenylhydrazine sulfate soln, 129, and proceed as directed in 130, beginning with the words "allow to stand for 48 hours."

132

#### SULFONAL<sup>132</sup>—TENTATIVE

Mix about 0.5 g of the sample with pure, clean sea sand and place the mixture in a Knorr tube containing a half-inch layer of asbestos. Using a bell jar and vacuum, extract the mixture with 10 portions of 10 cc each of ether, mixing the sample with the sand by means of a glass rod before each addition of ether. Collect the ether extractions in a tared flask, distil off the bulk of the ether, and allow the remaining solvent to evaporate spontaneously, rotating the flask to aid the evaporation. Dry the residue in a desiccator over H<sub>2</sub>SO<sub>4</sub> for 18 hours and weigh. Identify the residue by means of its melting point.

If desired, the extraction may be made in a suitable automatic apparatus (Fig. 51).

133

TRIONAL<sup>TM</sup>—TENTATIVE

Proceed as directed under 132.

134

STRAMONIUM OINTMENT<sup>TM</sup>—TENTATIVE

Introduce 25 g of the well-mixed ointment into a 250 cc separator fitted with a pledget of cotton packed loosely in the stem; add 100 cc of ether- $\text{CHCl}_3$  mixture (4 to 1) and shake vigorously until all the fats have been dissolved. Extract the alkaloids by shaking out with five successive 20 cc portions of dilute  $\text{H}_2\text{SO}_4$  (2% is satisfactory), allow to settle, and draw off the clear acid soln into a small separator containing 10 cc of ether. Wash each acid extraction successively

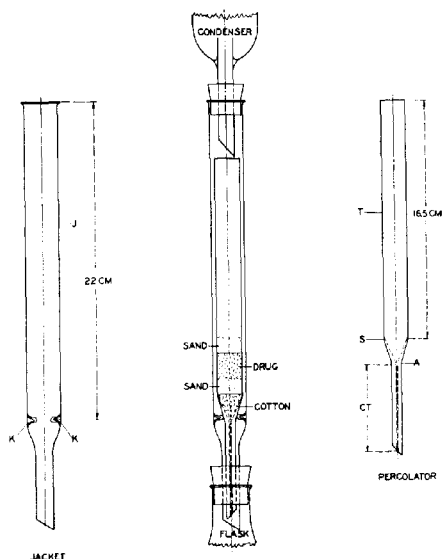


FIG. 51.—AUTOMATIC PERCOLATOR

thru this same 10 cc of ether and draw off the acid solns into another 250 cc separator. Make the combined acidified solns alkaline with ammonia water and extract the alkaloids completely by shaking out with 5 successive 25 cc portions of  $\text{CHCl}_3$ , allow to settle, and filter each portion thru cotton wetted with  $\text{CHCl}_3$  into a 250 cc beaker, finally washing the stem of the separator and the filter with a little  $\text{CHCl}_3$ . Evaporate the solvent carefully on a water bath with moderate heat to a volume of about 10 cc; add a measured excess (about 10 cc) of 0.02  $N$   $\text{H}_2\text{SO}_4$ , stir the mixture, and continue the evaporation until all the  $\text{CHCl}_3$  has been expelled. Add 20 cc of recently boiled, cooled  $\text{H}_2\text{O}$  and one drop of methyl red indicator and titrate the excess acid with 0.02  $N$   $\text{NaOH}$ . 1 cc of 0.02  $N$   $\text{H}_2\text{SO}_4$  = 0.00378 g of the alkaloids of stramonium leaves.

135

*Paraffin Method*

Weigh about 25 g of the ointment into a tall-form beaker. Add about 5 g of paraffin, 25 cc of 2%  $\text{H}_2\text{SO}_4$ , and 10 cc of ether. Warm gently on a steam bath until fluid, stirring the mixture thoroly. Continue this procedure until most of the ether has been evaporated. Place the beaker in an ice bath and allow to stand until cold. Make several holes in the paraffin layer with a stirring rod and filter the acid soln thru a pledget of cotton into a small separator. Wash the cake once with a small quantity of  $\text{H}_2\text{O}$ , filtering the washings thru the cotton into the separator. Wash the acid with 10 cc of ether and draw off into a 250 cc separator. Repeat the treatment with 4 successive portions of acid and ether, filtering each portion thru the cotton into the small separator and washing each extraction with the same 10 cc portion of ether. Combine the acidified extractions, make alkaline with 10%  $\text{NH}_4\text{OH}$ , and extract and titrate the alkaloids as in the preceding method.

NOTE: It is recommended that the ointment be transferred by means of a soft metal ointment tube or empty tooth paste tube, and weighed by difference. The assay can be hastened by centrifuging when instructions are given to let the mixture stand until it settles.

136

BELLADONNA OINTMENT<sup>u</sup>—TENTATIVE

Proceed as directed under 134 or 135. 1 cc of 0.02  $N$   $\text{H}_2\text{SO}_4$  = 0.00578 g of the alkaloids of belladonna leaves.

137

THEOBROMINE IN THEOBROMINE CALCIUM<sup>u</sup>—TENTATIVE

Dry about 0.5 g of the material at  $110^\circ$  to constant weight. Weigh 0.2 g of the dried substance into a glass-stoppered 100 cc volumetric flask, add 2 cc of glacial acetic acid, and warm on the steam bath. Add 10 cc of boiling  $\text{H}_2\text{O}$  and shake until soln has taken place, adding more boiling  $\text{H}_2\text{O}$  if necessary. Cool the soln to room temp. (The soln should be clear or nearly so.) Add 50 cc of 0.1  $N$  iodine, 20 cc of saturated salt soln and 2 cc of  $\text{HCl}$ . Shake well and make to volume with  $\text{H}_2\text{O}$ . Shake again and allow to stand overnight. Filter, discarding the first 10 cc of the filtrate. Titrate 50 cc of the filtrate with 0.1  $N$   $\text{Na}_2\text{S}_2\text{O}_3$ , using starch soln as indicator. 1 cc of 0.1  $N$   $\text{I}$  = 0.0045 g of theobromine,  $\text{C}_7\text{H}_5\text{O}_2\text{N}_4$ .

ARSENIC IN IRON-ARSENIC TABLETS<sup>u</sup>—OFFICIAL

138

## REAGENT

*Standard soln of potassium bromate (or of iodine).*—Standardize against pure  $\text{As}_2\text{O}_3$ . (The strength of this soln is a matter of choice. 0.5625 g of  $\text{KBrO}_3$  dissolved in  $\text{H}_2\text{O}$  and diluted to 1 liter will give a soln that is 0.02021  $N$ , 1 cc of which = 0.001 g of  $\text{As}_2\text{O}_3$ .)

139

## APPARATUS

Use either the Ramberg-Sjöström arsenic flask, which consists of a 300 cc Kjeldahl flask provided with a specially shaped outlet tube connected with the flask by means of a ground joint (A, Fig. 52), or a 300 cc Kjeldahl flask provided with an outlet tube, the internal diameter of the main part of which is about 13 mm and that of the contracted tip about 5 mm, connected with the flask by means of a rubber stopper (B, Fig. 52).

140

## DETERMINATION

Weigh and place in the flask 5-10 tablets or pills, add 10-15 cc of  $\text{H}_2\text{O}$ , and allow to soak for 30 min. Then add, in small portions at a time, 20 cc of fuming  $\text{HNO}_3$ ,

cooling if necessary to prevent loss by frothing. When the reaction has ceased, add carefully and in small portions at a time 25–28 cc of  $\text{H}_2\text{SO}_4$ . Place the flask in an inclined position on an asbestos mat and heat over a small flame. As soon as the greater part of the  $\text{HNO}_3$  has been driven off, and while still heating, drop in 8 cc of fuming  $\text{HNO}_3$  thru a suitably placed separator and heat over a larger flame until  $\text{SO}_2$  is evolved. If after cooling the precipitated sulfates are not colorless or pale yellow and are not free from gray or black particles, heat the contents of the flask further with an additional 10 cc of fuming  $\text{HNO}_3$ . (It is essential that all organic matter be destroyed.) To the cooled mixture add 30 cc of a saturated soln of  $\text{NH}_4$  oxalate; heat until fumes of  $\text{SO}_2$  are evolved and, to insure complete destruction of the oxalic acid, for 10 min. thereafter over a low flame; cool; and add while gently whirling the flask 20 cc of  $\text{H}_2\text{O}$ . Dry the neck of the flask over a small flame

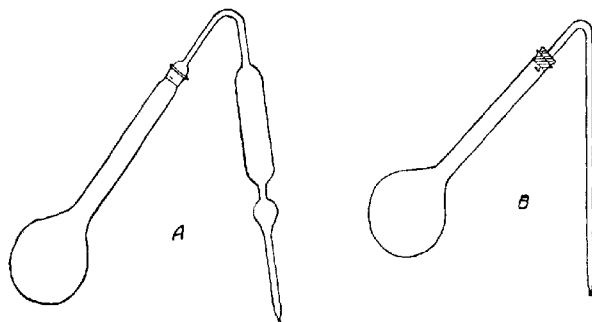


FIG. 52.—APPARATUS FOR THE DETERMINATION OF ARSENIC IN IRON-ARSENIC TABLETS

and add 30 g of  $\text{NaCl}$ , 5 g of  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  (or 1 g of hydrazine sulfate), 1 g of  $\text{NaBr}$ , and 25 cc of  $\text{HCl}$ . Mix the contents of the flask and connect the delivery tube. If the Ramberg-Sjöström apparatus is used, moisten the ground-glass joint with a drop of  $\text{H}_2\text{SO}_4$ . Fix the flask in an inclined position with the tip of the outlet tube about 1 cm under the surface of 150 cc of  $\text{H}_2\text{O}$  in an Erlenmeyer flask surrounded by ice or by cold  $\text{H}_2\text{O}$ . Distil at such a rate that the bend at the top of the tube comes warm in 4 min. and the lower end in about 8 min. from the time the heat is applied. Discontinue the distillation at the end of 10 min., but before removing the flame lift the distillation flask until the tip of the outlet tube is above the  $\text{H}_2\text{O}$  in the receiving flask. Let the outlet tube drain, remove the receiver, and either titrate with the standard soln of  $\text{KBrO}_3$ , using 2 drops of methyl orange indicator (the red color of the indicator at the end point may fade slowly, but the color should persist for at least 1 min. upon addition of another drop of the indicator); or nearly neutralize with  $\text{NaOH}$ , add 4–5 g of  $\text{NaHCO}_3$ , and titrate with the standard soln of  $\text{I}_2$ , using starch indicator, VI, 3(e).

#### 141

#### ARSENIC IN SODIUM CACODYLATE<sup>us</sup>—OFFICIAL

Transfer 0.2 g of the sample, accurately weighed, to a Kjeldahl flask. Add 10 g of  $\text{K}_2\text{SO}_4$ , 0.3 g of starch, and 20 cc of  $\text{H}_2\text{SO}_4$ . Digest over a low flame until frothing

has ceased. Continue the digestion 4 hours or until the mixture is colorless. Cool, dilute with  $H_2O$ , and transfer to a 500 cc Erlenmeyer flask. Add  $NaOH$  soln (1+1) slowly until alkaline to litmus paper and acidify with  $H_2SO_4$ . Place the flask in  $H_2O$  until thoroly cooled, add 5 g of  $NaHCO_3$ , and titrate with 0.1 N I soln. Conduct a blank, using the same quantities of reagents. 1 cc of 0.1 N I soln = 0.00375 g of As, or 0.008 g of anhydrous Na cacodylate.

142

ARSENIC IN IRON METHYLARSENATE<sup>20</sup>—OFFICIAL

Transfer a suitable quantity of the sample (0.2 g, if practicable) to a Kjeldahl flask. Add 10 g of  $K_2SO_4$ , 0.3 g of starch, and 20 cc of  $H_2SO_4$ . Digest over a low heat until frothing has ceased and continue the digestion over a slightly higher flame until the mixture is colorless. Cool, and add 20 cc of  $H_2O$ . Dry the neck of the flask over a small flame; cool the contents; and add 30 g of  $NaCl$ , 5 g of  $FeSO_4 \cdot 7 H_2O$ , 1 g of  $NaBr$ , and 25 cc of  $HCl$ . Then distil as directed under 140. Conduct a blank, using the same quantities of reagents.

BISMUTH COMPOUNDS IN TABLETS<sup>21</sup>—TENTATIVE

143

(Lead absent.)

Thoroly mix the sample and weigh 0.5 g into a 500 cc Kjeldahl flask. Ignite gently over a small flame, using a wire gauze under the flask, and increase the heat towards the end. Allow to cool, add 15–20 cc of  $HNO_3$ , evaporate to dryness, and ignite as before until yellow or orange  $Bi_2O_3$  is formed. Cool the residue, and dissolve in 10–15 cc of warm  $HNO_3$ , using a few cc of 3%  $H_2O_2$  if there is difficulty in obtaining a soln. Boil off the excess  $H_2O_2$  and wash into a 400 cc beaker with  $H_2O$ , rinsing the flask well. Dilute to about 200 cc, make just neutral to litmus with  $NH_4OH$ , and add 5 cc of  $HCl$ . Precipitate with  $H_2S$  completely.

Transfer the precipitate to a filter paper and wash once with  $HCl$  (5+200) and then several times with  $H_2O$ . Dissolve the precipitate of  $Bi_2S_3$  on a filter with hot  $HNO_3$  (1+2). A small residue of S (and mercuric sulfide if mercury salts are present) usually remains. Neutralize the filtrate with 10%  $NH_4OH$  and precipitate with 25 cc of a 20% soln of ammonium carbonate. Concentrate to about 150 cc (by boiling, if desired) and allow to stand on the steam bath for 1–2 hours. Collect the precipitate in a previously ignited weighed Gooch crucible, wash with a small quantity of  $H_2O$ , dry, ignite in a muffle at red heat, and weigh as  $Bi_2O_3$ .

144

CALCIUM GLUCONATE<sup>22</sup>—TENTATIVE

(Applicable to preparations the aqueous solns of which are neutral and do not contain salts of other optically active hydroxy acids.)

Weigh two 0.5 g portions of calcium gluconate or two 1 g portions of powdered tablets containing 50% or less of the salt. If chocolate or a fatty base is present, wash the samples several times on a hardened filter with absolute ether and warm the residue until the ether is driven off. Transfer each portion to a separate, 25 cc volumetric flask, add 15 cc of  $H_2O$ , and warm until the calcium salt is dissolved (there will be an undissolved residue in the case of samples containing cocoa). Cool to room temp. To one flask (No. 1) add 3.5 g of finely pulverized uranyl acetate, stopper, and place the mixture in a shaking machine for 1 hour (if agitation is not sufficiently vigorous, more than 1 hour's shaking may be required). Allow the other flask (No. 2) to stand. If the sample contains chocolate, add a little alumina cream **XXXIV**, 18(b), to each flask. Cool to 20°, make up the contents of flask No. 1 to volume with uranyl acetate soln (10 g shaken with 95 cc of  $H_2O$  until saturated, then

filtered), and flask No. 2 with  $H_2O$ . Filter, and polarize each soln in a 200 mm tube, using a 50 mm tube containing a 1.8% soln of  $K_2Cr_2O_7$  as a light filter. If the soln is too dark to read in the 200 mm tube, make the reading in a 100 mm tube and multiply the result by 2. If  $A$  = the rotation in  $^\circ V$ . of soln No. 2 and  $B$  = the rotation of soln No. 1, with 1 g samples the percentage of  $Ca(C_6H_{11}O_7)_2 = 4.34 (B - A)$ , and with 0.5 g samples the percentage of  $Ca(C_6H_{11}O_7)_2 = 8.52 (B - A)$ .

## 145

HYPOPHOSPHITES<sup>n</sup>—TENTATIVE

(Applicable in absence of phosphates; if phosphates are present, make suitable correction.)

(a) *Total hypophosphites*.—Place 25 cc of the sample in a 100 cc volumetric flask, dilute to mark, and mix thoroly. Pipet a 10 cc aliquot into a suitable flask. Add 25 cc of  $HNO_3$ , and boil on a hot plate until the volume is reduced to 2–3 cc; add 10 cc of  $HNO_3$ , and boil until volume is again reduced to 2–3 cc. Cool, and add 20 cc of  $H_2O$ . Add  $NH_4OH$  in slight excess, and barely dissolve the precipitate formed with a few drops of  $HNO_3$ , stirring vigorously. To the hot soln add 70 cc of molybdate soln, II, 7(a), for each 0.1 g of  $P_2O_5$  present. Digest at about  $65^\circ$  for 1 hour, and test for complete precipitation by the addition of more reagent to the clear, supernatant liquid. Filter, and wash with  $NH_4NO_3$  soln, II, 7(b).

Dissolve the precipitate on the filter with  $NH_4OH(1+1)$  and hot  $H_2O$  and wash into a beaker to a volume of not more than 100 cc. Neutralize with  $HCl$ , using litmus paper as an indicator. Cool, and from a buret add slowly (about 1 drop per second, stirring vigorously) 15 cc of magnesia mixture, II, 7(c), for each 0.1 g of  $P_2O_5$  present. After 15 min. add 12 cc of  $NH_4OH$  and allow to stand overnight. Filter, and wash the precipitate with dilute  $NH_4OH$ , II, 7(d), until the washings are practically free from chlorides. Dry, burn first at a low heat, and ignite to constant weight, preferably in an electric furnace, at  $950-1000^\circ$ . Cool, and weigh as  $Mg_2P_2O_7$ .  $Mg_2P_2O_7 \times 0.6379 = P_2O_5$ .

(b) *Calcium*.—Using the soln prepared as directed in (a), pipet a 20 cc aliquot into a 400 cc beaker and dilute to 100 cc. Add 2 cc of  $HCl$ , 15 cc of 10%  $NH_4C_2H_3O_2$  soln, and a slight excess of saturated  $(NH_4)_2C_2O_4$  soln. Heat to boiling and allow the precipitate to settle at a temp. just below boiling. Filter hot, wash with 1%  $NH_4C_2H_3O_2$  soln, dry, moisten with  $H_2SO_4$ , ignite gently, and weigh residue as  $CaSO_4$ .  $CaSO_4 \times 0.2944 = Ca$ .

## 146

IODINE<sup>14</sup>—TENTATIVE

Transfer a quantity of the sample that contains not more than 0.1 g of the iodide (0.05 g is ample) to a crucible, preferably nickel, and add 2 or 3 g of solid  $KOH$ . If the sample is a solid, add 10–15 cc of alcohol before adding the  $KOH$ . Dry and char thoroly. (Use as low a temp. as possible in order to prevent loss of iodide, in no event more than dull redness.) Extract the charred mass with hot  $H_2O$ , filter into an Erlenmeyer flask, and wash well with hot  $H_2O$ .

Neutralize the filtrate with  $H_2SO_4$  (1+1), make alkaline again, and add 1 cc of 4%  $NaOH$  soln in excess. Heat to boiling and add saturated  $KMnO_4$  soln slowly until the permanganate color remains after several minutes' boiling. Then add about 0.5 cc in excess, continue boiling about 5 min., and allow to cool. Add a few cc of alcohol and set on the steam bath. (The permanganate color should be bleached; if it is not, add a little more alcohol.) When the precipitate has settled, filter and wash with hot  $H_2O$ . After cooling, add 1–2 g of  $KI$  (crystals), acidify with  $HCl$ , and titrate with 0.1  $N$   $Na_2S_2O_3$  soln. 1 cc of 0.1  $N$   $Na_2S_2O_3 = 0.002768$  g of  $KI$ , 0.002498 g of  $NaI$  or 0.002116 g of  $I$ .

IODIFORM<sup>a</sup>—TENTATIVE

147

## REAGENTS

(a) *Ammonium thiocyanate soln.*—0.05 *N*. Standardize against 0.1 *N* AgNO<sub>3</sub> soln, using an equal volume of alcohol, and 3 cc of ferric ammonium sulfate soln as indicator.

(b) *Ferric ammonium sulfate indicator.*—Dissolve 8 g of Fe(NH<sub>4</sub>)(SO<sub>4</sub>)<sub>2</sub>·12H<sub>2</sub>O in 100 cc of H<sub>2</sub>O.

148

## DETERMINATION

Weigh accurately about 0.25 g of CHI<sub>3</sub> and transfer quantitatively to a 200 cc Erlenmeyer flask. Add 40 cc of alcohol, swirl gently until the CHI<sub>3</sub> is dissolved, filter if necessary, and immediately add 40 cc of 0.1 *N* AgNO<sub>3</sub> and 10 cc of HNO<sub>3</sub>. Swirl gently for about 5 min., allow to stand at room temp. for 2–3 hours, and then swirl occasionally as an aid in flocculating the AgI. Titrate the excess 0.1 *N* AgNO<sub>3</sub> with 0.05 *N* NH<sub>4</sub>CNS, using 3 cc of the ferric ammonium sulfate indicator. 1 cc of 0.1 *N* AgNO<sub>3</sub> = 0.01313 g of CHI<sub>3</sub>. Or, filter, collecting the AgI on a dried and accurately weighed Gooch crucible, wash with H<sub>2</sub>O and finally with alcohol, and dry to constant weight at about 125°. 1 g of AgI = 0.5590 g of CHI<sub>3</sub>.

149

IODIFORM OINTMENT<sup>a</sup>—TENTATIVE

Transfer approximately 2.5 g of the sample to a tared 50 cc beaker and weigh. Add 5 cc of CHCl<sub>3</sub>, stir gently with a glass rod, and transfer the bulk of the undissolved ointment and the CHCl<sub>3</sub> soln to a 250 cc flask having a ground-glass stopper. Add 5 cc of CHCl<sub>3</sub> to the ointment remaining in the beaker and stir until all the ointment is dissolved. Add the soln to the contents of the flask and finally wash the beaker three times, using not more than 5 cc of CHCl<sub>3</sub> each time, and add washings to contents of the flask. Or, weigh the sample in a small, tared glass capsule, drop capsule with contents into a 250 cc flask having a ground-glass stopper, and add not more than 20 cc of CHCl<sub>3</sub>. (Use glass capsule only in volumetric determination.) Swirl gently until all the ointment is dissolved. Add 40 cc of alcoholic 0.1 *N* AgNO<sub>3</sub> soln and swirl to wash down any iodoform that may adhere to the sides of the flask. Slowly add 10 cc of HNO<sub>3</sub> and allow to stand at room temp. for about 18 hours. Titrate the excess of alcoholic 0.1 *N* AgNO<sub>3</sub> soln with 0.05 *N* ammonium thiocyanate soln, 147(a), using 3 cc of ferric ammonium sulfate indicator, 147(b), shaking the mixture vigorously near the end of the titration.

For gravimetric determination use an ordinary Erlenmeyer flask in place of the flask having a ground-glass stopper. Weigh the ointment base into a 100 cc beaker and add CHCl<sub>3</sub>. When the ointment base has dissolved, filter thru a Gooch crucible, using suction. Wash beaker and crucible once with alcohol. Wash crucible several times with CHCl<sub>3</sub> without using suction. Collect the filtrate in an Erlenmeyer flask and add 40 cc of 0.1 *N* AgNO<sub>3</sub> soln and 10 cc of HNO<sub>3</sub> in small portions. Allow the mixture to stand 18 hours. Collect the AgI on a weighed Gooch crucible, using suction. Wash with H<sub>2</sub>O and then with alcohol. Finally wash repeatedly with CHCl<sub>3</sub> without suction. Dry the Gooch crucible and contents at about 125° to constant weight. 1 g of AgI = 0.5590 g of CHI<sub>3</sub>.

150

IODIFORM GAUZE<sup>a</sup>—TENTATIVE

Weigh in a tared weighing bottle with a ground-glass stopper a sample of iodoform gauze containing about 1 g of iodoform. (Iodoform gauze is usually moist and loses

weight rapidly when exposed to air.) Transfer to a 150 cc beaker, add about 75 cc of alcohol, and stir until the iodoform is dissolved. Filter into a 200 cc volumetric flask, draining the alcoholic soln with the aid of pressure upon the gauze. Wash 4 or 5 times, using 25 cc of alcohol each time, filter washings, and finally make up to volume with alcohol. Pipet a 40 cc aliquot into a 200 cc Erlenmeyer flask and immediately add 40 cc of 0.1 *N* AgNO<sub>3</sub> and 10 cc of HNO<sub>3</sub>. Proceed as directed under 149, beginning with "allow to stand at room temp."

#### MERCUROUS CHLORIDE (CALOMEL) IN TABLETS<sup>71</sup>—OFFICIAL

151

##### REAGENT

*Standard iodine soln.*—Dissolve about 14 g of I in a soln containing 18 g of KI in 100 cc of H<sub>2</sub>O and dilute to 1 liter. Standardize this soln against standard Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> soln, 3(b).

152

##### DETERMINATION

Count and weigh a representative number of tablets. Pulverize a quantity of tablets and weigh accurately a sufficient portion of the well-mixed sample to represent 0.19–0.26 g (3–4 grains) of calomel. Transfer to a 200 cc glass-stoppered Erlenmeyer flask, add about 50 cc of H<sub>2</sub>O, acidify with acetic acid, and after the soluble fillers have dissolved decant with the aid of suction thru a tightly packed asbestos mat placed on the plate of a Caldwell crucible. Wash once with H<sub>2</sub>O by decantation, then successively with alcohol and ether. Transfer the removable plate holding the mat and insoluble material to the original flask, washing into the flask any insoluble material adhering to the sides of the crucible. Add 2.5 g of KI, 10 cc of H<sub>2</sub>O, and then 30 cc of standard I soln, 3(c). Allow the mixture to stand, with frequent and fairly vigorous agitation, for about 1.5 hours, or until soln of the calomel is complete. Titrate with the standard thiosulfate soln, 3(b), and add about 1 cc in excess. Then titrate back with the standard I soln, using starch indicator, VI, 3(e), until a permanent blue color is obtained. 1 cc of 0.1 *N* I soln = 0.02361 g of calomel.

153

#### CALOMEL IN CALOMEL OINTMENT<sup>72</sup>—TENTATIVE

Weigh accurately about 1 g of the ointment, transfer to a 250 cc glass-stoppered Erlenmeyer flask, and treat with about 50 cc of CHCl<sub>3</sub>. When the base is dissolved, decant thru a dry, closely packed asbestos mat in a Caldwell crucible, using light suction. Wash the flask and contents several times with 20 or 30 cc portions of CHCl<sub>3</sub>, decanting thru the crucible. Allow any residual CHCl<sub>3</sub> in the flask to evaporate and transfer the asbestos mat and contents to the flask, wiping the sides of the crucible and the mouth of the flask with a damp piece of filter paper and adding it to the contents of the flask. Then add 2.5 g of KI and 50 cc of 0.1 *N* I soln, stopper, and mix the contents well. Allow the flask to stand about 1.5 hours or until soln of the calomel is complete, agitating it frequently and fairly vigorously. Titrate with 0.1 *N* Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>, adding 1 or 2 cc in excess and using starch as indicator. When all traces of I have disappeared, titrate back with the standard I soln until a blue color is obtained. 1 cc of 0.1 *N* I = 0.02361 g of calomel.

154

#### MERCUROUS IODIDE IN TABLETS<sup>73</sup>—TENTATIVE

Count and weigh a representative number of tablets. Pulverize a quantity of tablets and weigh accurately a sufficient portion of the well-mixed sample to represent 0.19–0.26 g (3–4 grains) of HgI<sub>2</sub>. Transfer the sample to a 200 cc glass-stoppered flask, add about 50 cc of H<sub>2</sub>O, acidify with acetic acid, and after the soluble fillers



have dissolved, decant with the aid of suction thru a tightly packed asbestos mat placed on the plate of a Caldwell crucible. Wash once with  $H_2O$  by decantation, then successively with alcohol and ether. Transfer the removable plate holding the mat and insoluble material to the original flask, washing into the flask any insoluble materials adhering to the sides of the crucible. Add 2.5 g of KI and 30 cc of standard I soln, 3(c). Allow the mixture to stand, with frequent and fairly vigorous agitation, for about 1.5 hours, or until soln of the  $HgI$  is complete. Titrate with standard thio-sulfate soln, 3(b), and add 1 or 2 cc in excess. When all traces of I have disappeared, titrate back with standard I, using starch indicator. 1 cc of 0.1  $N$  I soln = 0.03275 g of  $HgI$ .

NOTE.—Some commercial tablets are difficult to filter without loss of  $HgI$  thru the asbestos mat. A few drops of alumina cream, XXXIV, 18(b), washed free from  $NH_3$ , placed on the mat before filtration is started, satisfactorily prevents loss, tho it retards the filtration.

#### 155 TESTS FOR PURITY OF MERCUROCHROME<sup>®</sup>—TENTATIVE

(a) Acidify a portion of the mercurochrome soln with 10%  $H_2SO_4$  and filter off the precipitate. The filtrate is colored only slightly yellow.

(b) Pass  $H_2S$  into a portion of the filtrate. No precipitate or coloring occurs.

(c) Add a few cc of 10%  $HNO_3$  to another portion of the filtrate and add  $AgNO_3$  soln. No precipitate forms.

#### 156 TOTAL SOLIDS IN MERCUROCHROME SOLUTION<sup>®</sup>—TENTATIVE

Pipet 10 cc of the mercurochrome soln into a tared, extra-wide-form weighing bottle and evaporate to dryness on a steam bath. Allow to dry overnight in an  $H_2SO_4$  desiccator in the open bottle.

#### 157 MERCURY IN MERCUROCHROME<sup>®</sup>—TENTATIVE

Pipet 10 cc of an approximately 2% soln of the mercurochrome into a 500 cc tall-form beaker and evaporate to dryness on the steam bath (or weigh accurately about 0.2 g of the powder). Dissolve the residue in 4 cc of  $H_2O$  and add slowly, with constant mixing, 10 cc of  $H_2SO_4$ . Incline the beaker and add cautiously small portions of  $KMnO_4$  (finely pulverized), mixing after each addition, until considerable excess has been added, as indicated by the deep purple color of the mixture. Allow to stand 30 min., occasionally mixing, at the end of which time the mixture should still retain its purple color. Add 100 cc of  $H_2O$  and mix thoroly. Then add small portions of oxalic acid (finely pulverized), mixing after each addition, until the soln is clear. Filter thru a small filter into a 400 cc beaker, wash the original beaker, filter until the filtrate measures approximately 200 cc, and pass  $H_2S$  thru the soln for 20 min. Warm on a steam bath until the precipitate of  $HgS$  settles quickly after stirring, and again pass  $H_2S$  thru the warm soln for 5 min. Filter the soln immediately into a weighed Gooch crucible, and wash the precipitate on the filter well with  $H_2O$ , three times with the alcohol, and then with 4 or 5 portions of  $CCl_4$  or  $CS_2$  to remove any sulfur that may be present, allowing the liquid to run thru the crucible without suction, and finally wash with ether. Dry the precipitate to constant weight at  $100^\circ$ , and weigh as  $HgS$ . Test the dried precipitate qualitatively for  $Hg$  and other heavy metals. If any difficulty is experienced by the slow filtration during the washing with  $H_2O$ , allow the precipitate to drain and wash once with alcohol, then continue as directed.

Weight of  $HgS \times 0.8622$  = the equivalent weight of  $Hg$

158

MERCURY IN MERCURIAL OINTMENT<sup>13</sup>—TENTATIVE

After mixing the ointment thoroly with a glass rod, avoiding contact with metals, weigh 1 g of the material into an Erlenmeyer flask. Add 20 cc of  $H_2O$  and 20 cc of  $HNO_3$  and heat gently over a small flame until red fumes cease to evolve. Cool, and decant the aqueous soln from the ointment base into a separator. Wash the ointment base with 50 cc of boiling  $H_2O$ , cool, and decant into the separator. Repeat the washing until all the Hg is removed. Shake the combined solns in the separator with 50 cc of ether. Transfer the aqueous soln to an Erlenmeyer flask. Wash the ether three times with 10 cc portions of  $H_2O$  until the Hg is removed, adding the washings to the flask. Add 3 cc of ferric ammonium sulfate soln, 147(b), and titrate with 0.1  $N$   $NH_4CNS$ . 1 cc of 0.1  $N$   $NH_4CNS$  = 0.01003 g of Hg.

NITRITES IN TABLETS<sup>14</sup>—TENTATIVE

159

## PREPARATION OF SAMPLE

Count and weigh a suitable number of tablets to ascertain the average weight. Reduce to a fine powder, mix thoroly, and keep in a tightly stoppered bottle.

160

## DETERMINATION

Transfer to a 100 cc volumetric flask a quantity of the powdered sample equivalent to about 1 g of  $NaNO_2$ , add  $H_2O$  to mark, and mix thoroly. Filter thru a dry filter, rejecting the first 10 cc. Transfer a 50 cc aliquot to a 200 cc volumetric flask. Add in order 10 cc of saturated  $KClO_3$  soln, then slowly, with shaking, 10 cc of  $HNO_3$  soln (1+1), and allow to stand 30 min. Add 50 cc of 0.1  $N$   $AgNO_3$  soln and make up to mark with  $H_2O$ . After mixing thoroly, filter thru a dry filter, rejecting the first 20 cc of the filtrate. To 100 cc of the filtrate add 2 cc of ferric ammonium sulfate soln, 147(b), and titrate with 0.05  $N$  ammonium thiocyanate soln, 147(a). Make a blank determination, and correct if necessary.

1 cc of 0.1  $N$   $AgNO_3$  soln = 0.020703 g of  $NaNO_2$

NOTE.—Correct for any chloride that may be present in sample. If a large quantity of insoluble excipient is present, pipet 100 cc of  $H_2O$  into a flask with the powdered sample in order to avoid any error in volume.

161

PHENOLSULFONATES<sup>15</sup>—TENTATIVE

Dissolve the sample (equivalent to about 0.8 g of phenolsulfonate) in about 30 cc of  $H_2O$  and add 5 cc of  $HCl$ . Titrate with 0.4  $N$   $Br$  (11.134 g of  $KBrO_3$  + 50 g of  $KBr$  diluted to 1 liter with  $H_2O$ , standardized against 0.1  $N$   $Na_2S_2O_3$ . The bromine will be absorbed very rapidly at first, but as the titration proceeds the absorption becomes slower and slower.) Titrate as far as possible with no other indicator than the fading of the bromine yellow. (Usually this will be within about 1–4 cc of the end point.) Then use methyl orange (0.1%), dropwise, adding no new indicator until the previous drop has practically faded. After adding bromine soln, wait a sufficient time for the absorption of the bromine before adding more methyl orange (10 seconds at first, 15 seconds at end of titration), because in the presence of dibromophenolsulfonic acid the action of bromine on methyl orange is much slower than normal. The end point is reached when, after waiting 15 seconds for the absorption of the last drop of bromine and adding a drop of methyl orange, the latter fades very appreciably in 10 seconds. It is always best, after the methyl orange has faded, to add another drop to be sure that the first drop was not added too soon.

In using 0.1 *N* bromine, draw the indicator by dropping from a 10 cc graduated cylinder. If less than 1 cc of indicator is used, make no correction; if more, subtract 0.5 cc of 0.1 *N* Br for each cc of indicator.

1 cc of 0.4 *N* bromine = 0.023213 g of sodium phenolsulfonate;

1 cc of 0.1 *N* bromine = 0.0058 g of sodium phenolsulfonate.

#### SILVER PROTEINATES<sup>a</sup>

162

##### *Acidity or Alkalinity—Tentative*

Dialyze 1 g of the sample as directed under 164 and titrate a portion of the clear soln representing 0.5 g of the sample with either 0.02 *N* HCl or 0.02 *N* NaOH soln, as required, using phenolphthalein indicator. Calculate acidity as percentage of HCl and alkalinity as percentage of NaOH.

163

##### TOTAL SILVER<sup>a</sup>—OFFICIAL

Place 1 g, accurately weighed, in a 500 cc Kjeldahl flask; add 15 cc of H<sub>2</sub>SO<sub>4</sub> and then 10 cc of HNO<sub>3</sub>; place on a steam bath for a few min., with occasional rotation, to insure a homogeneous mixture; and boil to white fumes. Add more HNO<sub>3</sub>, boil again to a clear colorless soln, and cool. Add 100 cc of distilled H<sub>2</sub>O and boil until free of nitrogen oxides. Cool, dilute to 300 cc, add 5 cc of HNO<sub>3</sub> and 5 cc of ferric ammonium sulfate soln, 147(b), and titrate with 0.1 *N* ammonium thiocyanate. 1 cc of 0.1 *N* NH<sub>4</sub>CNS = 0.010788 g of Ag.

#### 164 DETECTION AND ESTIMATION OF IONIZABLE SILVER COMPOUNDS<sup>a</sup>—OFFICIAL

Weigh a strip of commercial dialyzing tubing 55 mm wide and about 1 foot long, wet with distilled H<sub>2</sub>O until uniformly pliable, shake free of adhering H<sub>2</sub>O, and partially dry by rolling in a clean paper towel. Reweigh while still moist and place in a 250 cc beaker. (Sheets of dialyzing parchment paper may be used in place of tubing.) Over one end of a glass tube 10 cm long and approximately 2.5 cm in diameter, fold and secure by means of a rubber band a square piece of parchment paper in the form of a sack of sufficient size to hold the sample soln. (Dialyzing material should be kept in a humid container to prevent breaking when handled.) Weigh 1 g of the sample, dissolve in 15 cc of H<sub>2</sub>O, and transfer to the dialyzing tube. Calculate, and add sufficient H<sub>2</sub>O to the beaker to make a total of 100 cc. (This insures 20 cc in the dialyzing tube and 80 cc in the beaker.) Adjust the tubing to form a "U" in the beaker, cover with a watch-glass, and place in a cool dark closet for 24 hours.

(a) *Qualitative Test*.—Test a few cc of the clear, colorless soln from the beaker for Ag ions by the addition of a few drops of 10% HCl and a trace of HNO<sub>3</sub>.

(b) *Quantitative Method*.—If Ag ions are present, remove 50 cc of the clear, colorless soln from the beaker (representing 0.5 g of sample), dilute to 100 cc, and add 2 cc of Fe NH<sub>4</sub>(SO<sub>4</sub>)<sub>2</sub>, 147(b), and the same quantity of colorless HNO<sub>3</sub>. Titrate with 0.01 *N* NH<sub>4</sub>CNS soln and calculate to percentage by weight of ionizable Ag. 1 cc of 0.01 *N* NH<sub>4</sub>CNS = 0.0010788 g of Ag.

#### OIL OF CHENOPODIUM<sup>a</sup>—TENTATIVE

165

##### REAGENTS

(a) *Standard ferric ammonium sulfate soln*.—Dissolve 39.214 g of pure, crystallized ferrous ammonium sulfate, Fe(NH<sub>4</sub>)<sub>2</sub>(SO<sub>4</sub>)<sub>2</sub>·6H<sub>2</sub>O, in 200 cc of H<sub>2</sub>O in a liter flask, add 30 cc of H<sub>2</sub>SO<sub>4</sub>, and mix well. Weigh exactly 3.16 g of KMnO<sub>4</sub>, dissolve

the salt in 200 cc of warm  $H_2O$ , and slowly add to the soln in the flask, with stirring. The permanganate soln should be just sufficient to oxidize the iron salt, but it is well to add the last few cc in small portions. Cool the soln and dilute it to 1 liter with  $H_2O$ .

(b) *Standard titanium trichloride soln.*—Add 100 cc of commercial 15–20%  $TiCl_3$  soln to 200 cc of  $HCl$ , boil for 1 min., cool, and dilute to 4500 cc with  $H_2O$ . Place the soln in a container with  $H$  atmosphere provision and allow to stand for 2 days for absorption of residual  $O$ . Preserve the  $TiCl_3$  soln in an atmosphere of  $H$  (XXI, Fig. 22), taking care to have all the joints air-tight, and covering the stoppers (preferably countersunk) with suitable wax. Standardize by titrating 20 cc of the ferric ammonium sulfate soln against the  $TiCl_3$  soln in a protective stream of  $CO_2$ , using 1 cc of 5%  $NH_4CNS$  soln as an indicator. 1 cc of 0.1  $N Fe_2(SO_4)_3 = 0.01545$  g of  $TiCl_3$ .

## 166

## DETERMINATION

Weigh 1 cc of the oil in a 100 cc volumetric flask and dilute to volume with 95% alcohol. Place 50 cc of the  $TiCl_3$  soln in an Erlenmeyer flask thru which a current of  $CO_2$  is passing. Fit the flask with a Bunsen valve, add 10 cc of the diluted soln of the oil, close the flask (with the Bunsen valve), and heat the contents almost to boiling for 2 min. (Prolonged heating has no effect if the contents are not boiled vigorously.) If the pale violet color of the  $TiCl_3$  disappears, add more of the reagent to insure an excess. (The formation of a white precipitate of titanate does not interfere with the determination.) Add 1 cc of a 5% soln of  $NH_4CNS$  and titrate back the excess of  $TiCl_3$  with the ferric ammonium sulfate soln in a  $CO_2$  atmosphere until a faint, permanent, brownish red color is obtained.

Subtract the quantity of ferric ammonium sulfate used, expressed in equivalent mg of  $TiCl_3$ , from the number of mg of  $TiCl_3$  taken. The difference is the number of mg of  $TiCl_3$  oxidized by the oil taken. Convert the mg of  $TiCl_3$  oxidized into ascaridole by dividing by the factor 1.284 (1 g of ascaridole is reduced by 1.284 g of  $TiCl_3$ ).

EXAMPLE: 0.9600 g of oil was made up to 100 cc and a 10 cc aliquot was heated with 50 cc of the  $TiCl_3$  soln (1 cc containing 0.0034 g of the salt). It then required 5.9 cc of the reagent, each cc equivalent to 0.01545 g of  $TiCl_3$ , to titrate back the soln. The grams of  $TiCl_3$  oxidized is numerically equal to  $(50 \times 0.0034) - (5.9 \times 0.01545)$ , or 0.07885. The weight of oil in the aliquot was 0.0960 g. Hence the

$$\text{percentage of ascaridole} = \frac{0.07885 \times 100}{0.096 \times 1.284} = 72.1\%$$

## BIOASSAY OF DRUGS

## MYDRIATICS AND MYOTICS

*Cat-Eye Method*<sup>89</sup>—Official

## 167

## APPARATUS

(a) *Mohr pipets.*—1 cc, graduated in 0.1 cc, with slender tips that deliver exactly 0.05 cc per drop.

(b) *N-filled electric lamps.*—100-watt or equally intense illumination.

## 168

## ANIMALS

*Adult cats.*—In good physical condition, weighing over 1500 g, and accustomed to being handled.

## 169

## PREPARATION OF SAMPLE

Dissolve, in approximately neutral distilled  $H_2O$ , a representative number of tablets, or a sufficient quantity of powder, to make a soln containing 1 mg of the alkaloid per cc of soln. If the alkaloids themselves are taken, add the equivalent quantities of acid to convert them into the corresponding salts. Add 2 drops of approximately 0.02 *N* acid per 50 cc of soln.

For great accuracy, the results of chemical assay upon the sample should be followed in the preparation of solns; when such accuracy is unnecessary, the declaration of strength on the label may be accepted as the basis for the preparation of the soln.

One drop of the respective concentrations of the following drugs is the minimum effective dose:

MYDRIATICS	mg per liter
Atropine.....	12
Hyoseyamine.....	4
Scopolamine.....	0.4
Homatropine.....	200
Cocaine.....	60
Euphthalmin.....	50,000
Ephedrine (alkaloid).....	2,500
Ephedrine salt (or synthetic).....	50,000
Pseudoephedrine (alkaloid).....	2,500
Pseudoephedrine (salt).....	80,000
MYOTICS	
Pilocarpine.....	25,000
Physostigmine (eserine).....	10
Arecoline.....	10,000

## 170

## DETERMINATION OF CAT'S THRESHOLD

Place a cat about 1 foot from a 100-watt electric lamp, and determine the maximum contractility of its pupils under this condition. Drop 0.05 cc of the freshly prepared standard mydriatic soln, obtained by diluting the 1 mg-per-cc soln, into the outer margin of one eye, leaving the other eye untreated as a control. Compress the inner canthus, while opening and closing the lids, until the fluid has apparently disappeared (10-30 seconds). Return cat to cage.

One and two hours after application (for atropine, 3 and 4 hours also), place cat under the same conditions, and note any differences in diameter between the pupils of the treated and the untreated eyes. (A satisfactory reaction is produced when the pupil of the treated eye is just perceptibly wider (0.5-1.0 mm) than the pupil of the untreated eye.) Do not use the same eye for another assay for at least 24 hours.

If the concentrations given fail to produce a satisfactory reaction, repeat the test with a stronger or weaker soln until the minimum effective concentration is found. (This concentration may vary somewhat for different cats, but it is essentially constant for the same cat.)

## 171

## BIOASSAY OF UNKNOWN SOLUTIONS

Dilute the 1 mg-per-cc soln to be tested to the minimum effective concentration for the cats to be used, and drop 0.05 cc of this dilution into one eye of the cat, following the same procedure as in the determination of the minimum effective concentration. Also prepare stronger and weaker solns and apply to one eye of each of

the other cats used. Test various concentrations until one is obtained that produces satisfactory mydriasis of the same degree as the standard soln when tested on two or more cats.

To obtain the mg of alkaloid present in each cc of the original soln, multiply the mg per cc found to be the cat's minimum effective concentration by the dilution employed. Knowing that the original soln was made to contain 0.001 g of alkaloid per cc, calculate the quantity of mydriatic present, and express as percentage of the total alkaloid.

#### ASSAY OF ERGOT<sup>®</sup>—TENTATIVE

(Applicable to alkaloids of ergotoxine-ergotamine group.)

172

##### REAGENTS

- (a) *Menstruum I.*—Mix 20 cc of HCl with 490 cc of alcohol and 490 cc of H<sub>2</sub>O.
- (b) *Menstruum II.*—Diluted alcohol U.S.P.
- (c) *Locke-Ringer soln modified.*—Omit the use of MgCl<sub>2</sub> in the Locke-Ringer soln. U.S.P. XI.

173

##### APPARATUS

Use an isolated organ bath similar to that described in the U.S.P. XI under Liquor Pituitarii Posterii, modifying it by having two glass chambers for the isolated tissues instead of one. Fix two levers, one above each chamber to write on the recording drum. The magnification of the two levers should be approximately equal.

174

##### PREPARATION OF SAMPLE

Pack the ergot, recently ground to a No. 20 powder, in a cylindrical percolator, and slowly percolate with purified petroleum ether until a few drops of the percolate leave no greasy stain when evaporated from filter paper. Reject the soln, remove the drug from the percolator, and dry it by exposure to the air.

Moisten the defatted drug with a 5% soln of NaHCO<sub>3</sub> and allow it to stand in a cold room for 2-4 hours. Pack in a percolator, add more bicarbonate soln and allow to macerate for 16-24 hours in a cold room. Percolate slowly with H<sub>2</sub>O until the percolate is found by physiological tests to be practically free from amines. Allow to drain completely, remove all but the lowest inch of marc from the percolator, and remove H<sub>2</sub>O from it by strong expression or by centrifuging. Moisten the drug with a small quantity of Menstruum I. Repack the drug in the percolator and macerate overnight in a cold room. Add the remainder of Menstruum I, and when this has just disappeared from the surface gradually add Menstruum II, constantly maintaining a stratum of liquid above the drug. When the liquid begins to drop from the percolator, close the lower orifice, and, having closely covered the percolator, macerate for 48 hours (in a cold room), and then allow the percolation to proceed slowly, gradually adding Menstruum II until the drug is exhausted. Reserve the first 850 cc of the percolate, recover the alcohol from the remainder of the percolate, and concentrate the residue to a soft extract at a temp. not exceeding 60° (preferably in a vacuum distillation apparatus). Dissolve the extract in the reserved portion, mix thoroly, and assay a portion by the method given below. From the results thus obtained adjust the volume of the finished fluidextract by the addition of Menstruum II to make it conform to the required biological standard.

175

##### THE TEST

Use nonpregnant female rabbits weighing 2 kg or more, and which are at least 3 weeks past parturition. Kill the animal by a blow on the head, cut its throat, and

then suspend by the hind legs until hemorrhage ceases. Remove the uterus. Cut a piece about 1 cm in length from one uterine horn. The remainder of the uterine horns may be placed between pieces of cotton wool dampened with the modified Locke-Ringer's soln and kept, properly covered, at a temp. of 40-50°F for any subsequent tests within the next 2 days. Place the piece of uterine horn on the convex surface of a watch-glass and cut it open along the line of mesometric attachment. Unfurl, and cut away the sides so that a piece 8-10 mm wide farthest from the mesometrium remains. Divide this piece longitudinally into two equal parts and suspend each in one of the glass containers of the bath holding the Locke-Ringer soln. Weight the recording levers so as to induce relaxation of the muscle. To each bath add 0.02 mg of epinephrine (as the hydrochloride in soln). If necessary, increase simultaneously the dose in each bath by increments of 0.01 or 0.02 mg until a contraction which is maintained 2-3 min. is produced. Adjust the weights on the levers so that the extent of contraction in each bath is similar and conspicuously greater than any spontaneous contractions. Spontaneous contractions increase in amplitude as the muscle remains in the bath so that weighting should as a rule be greater than at first seems necessary. At a noted time add to one bath a dose of a specific alkaloid. (This may be 0.4 cc of a soln of ergotamine tartrate or ergotoxine phosphate, 1 in 30,000. The concentration may vary from 1 part in 10,000 to 1 part in 200,000, depending upon the response obtained in the muscle.) Thirty seconds later add to the other bath 0.4 cc of the unknown soln diluted approximately 30 times; 10 min. from the time of the additions to each bath add epinephrine to each bath in the dosage found to give a previously satisfactory contraction. Note the contraction produced on each muscle. If the contraction has been reduced to the same extent in both baths the concentration of the specific alkaloid in each bath is the same. If the contractions are not the same, repeat the procedure, using fresh uterine strips, the same dose of epinephrine and larger or smaller doses of the ergot preparation as indicated by the previous test.

#### MICROCHEMICAL TESTS FOR ALKALOIDS—TENTATIVE OR OFFICIAL

##### 176

##### REAGENTS

- (a) *Sodium carbonate soln.*—Dissolve 5 g of  $\text{Na}_2\text{CO}_3 \cdot \text{H}_2\text{O}$  in 100 cc of  $\text{H}_2\text{O}$ .
- (b) *Kraut's reagent.*—Dissolve 8 g of  $\text{Bi}(\text{NO}_3)_3 \cdot 5\text{H}_2\text{O}$  in 20 cc of  $\text{HNO}_3$ , sp. gr. 1.18. Dissolve 27.2 g of KI in 50 cc of  $\text{H}_2\text{O}$ . Mix the solns and dilute to 100 cc.
- (c) *Wagner's reagent.*—Dissolve 1.25 g of  $\text{I}_2$  and 2 g of KI in 5 cc of  $\text{H}_2\text{O}$  and dilute to 100 cc.
- (d) *Potassium iodide soln.*—Dissolve 5 g of KI in 100 cc of  $\text{H}_2\text{O}$ .
- (e) *Mercuric chloride soln.*—Dissolve 5 g of  $\text{HgCl}_2$  in 100 cc of  $\text{H}_2\text{O}$ .
- (f) *Sodium benzoate soln.*—Dissolve 5 g of Na benzoate in 100 cc of  $\text{H}_2\text{O}$ .
- (g) *Platinic chloride soln.*—Dissolve 5 g of  $\text{H}_2\text{PtCl}_6$  in 100 cc of  $\text{H}_2\text{O}$ .
- (h) *Disodium phosphate soln.*—Dissolve 5 g of  $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$  in 100 cc of  $\text{H}_2\text{O}$ .
- (i) *Marmé's reagent.*—Dissolve 3 g of  $\text{CdI}_2$  in 18 cc of  $\text{H}_2\text{O}$  containing 6 g of KI.
- (j) *Gold chloride soln.*—Dissolve 1 g of gold chloride in 20 cc of  $\text{H}_2\text{O}$ .
- (k) *Mercuric chloride-sodium chloride soln.*—Dissolve 5 g of  $\text{HgCl}_2$  and 0.75 g of NaCl in 100 cc of  $\text{H}_2\text{O}$ .
- (l) *Zinc chloride soln.*—Dissolve 5 g of  $\text{ZnCl}_2$  in 100 cc of  $\text{H}_2\text{O}$ .
- (m) *Ammoniacal silver nitrate.*—Mix 5 cc of 2%  $\text{AgNO}_3$  soln and 5 cc of 10% ammonium hydroxide soln.
- (n) *Mayer's reagent (mercuric-potassium iodide soln).*—Dissolve 1.36 g of  $\text{HgCl}_2$  in 60 cc of  $\text{H}_2\text{O}$  and 5 g of KI in 10 cc of  $\text{H}_2\text{O}$ ; mix these two solns and dilute to 100 cc.

177

## PREPARATION OF SAMPLES

(a) *Controls*.—Dissolve 1 mg of the pure alkaloidal salt in 2 drops of  $H_2O$  to make an approximately 1–100 soln.

(b) *Alkaloids in compounds*.—Separate the alkaloid in pure form by extracting it from ammoniacal soln with a suitable immiscible solvent, and evaporate the solvent. To 1 mg of the residue add, dropwise, 0.1 *N* HCl, avoiding an excess of acid, and dilute with  $H_2O$ , if necessary, to approximately the same alkaloidal concentration as is specified in (a).

(c) *Hypodermic tablets*.—Dissolve a portion of a tablet in  $H_2O$  and dilute with  $H_2O$  to approximately the same alkaloidal concentration as is specified in (a).

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## IDENTIFICATION

Place a drop of the alkaloidal soln on a clean glass slide, add a drop of reagent by means of a clean glass rod, and without stirring or covering examine under the microscope, using low power. A magnification of 100–150 is suitable. Note the kind of crystals formed and compare their characteristics with the descriptions given and also with a control (see table under 179).

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*Characteristics of Microchemical Tests for Alkaloids*

ALKALOID	REAGENT	DESCRIPTION OF CRYSTALS
Aconitine <sup>21</sup>	Sodium carbonate	In 1:3000 soln heated to 50° in test tube. Small transparent hexagonal plates, also rods in contact
Arecoline <sup>21</sup>	Kraut's	Red, rhombic crystals
Atropine <sup>22</sup> (official first action)	Wagner's	Small dark rods and triangular plates form in great numbers, singly and in groups
Brucine <sup>23</sup>	Potassium iodide	Long masses of transparent, rectangular plates; also rosettes of thin plates
	Mercuric chloride	Small, dense rosettes
Caffeine <sup>23</sup>	Mercuric chloride	Clusters of long, radiating, needle-shaped crystals
Cinchonidine <sup>24</sup>	Sodium benzoate	Rosettes and sheaves of needles spreading to large size
	Platinic chloride	Rosettes of transparent plates
	Sodium carbonate	Spherical crystals, but not needles as in cinchonine
Cinchonine <sup>24</sup>	Sodium carbonate	Dark rosettes, composed of radiating needles, form immediately
	Disodium phosphate	Similar to crystals formed by sodium carbonate, but more burr-shaped
Cocaine <sup>25</sup>	Platinic chloride	Delicate, feathery crystals, later becoming heavier in structure
Codeine <sup>25</sup>	Marmé's	Silvery circular masses, crystallizing into dark rosettes of irregular outline



*Characteristics of Microchemical Tests for Alkaloids—Continued*

ALKALOID	REAGENT	DESCRIPTION OF CRYSTALS
Codeine <sup>95</sup>	Wagner's	Heavy, red-brown precipitate, crystallizing very slowly in yellow blades extending in branches (never red)
Ephedrine <sup>96</sup>	Kraut's	Long, brown radiating and interlacing needles
Heroin <sup>97</sup> (official)	Platinic chloride	Spherical clusters of golden yellow needles form slowly around a nucleus; cluster disintegrates on standing
Homatropine <sup>98</sup>	Gold chloride	Green-gold blades, often with pointed ends and united in pairs (1:200); surfaces appear etched on long standing
Hyoseyamine <sup>99</sup>	Gold chloride	Thin, transparent, nearly colorless irregular plates, often curved. Crystals form slowly in 1:100 to 1:200 soln. Shaking the slide aids crystallization
Morphine <sup>100</sup> (official)	Marmé's	Silvery, gelatinous precipitate, crystallizing in dense masses of fine needles
	Wagner's	Small drop of reagent produces heavy, red-brown precipitate, slowly crystallizing in shining red, overlapping plates extending in branches
Nicotine <sup>101</sup>	Mercuric chloride	Radiating transparent blades form in presence of slight excess of H <sub>2</sub> SO <sub>4</sub> ; feather-like blades form in the presence of HCl
	Mercuric chloride-sodium chloride	Radiating transparent blades
Papaverine <sup>102</sup>	Zinc chloride	Thin rectangular plates in excess HCl
Pilocarpine <sup>103</sup> (official first action)	Platinic chloride	Crystals form slowly; layers of thin, yellow, triangular plates of delicate structure
Procaine hydrochloride <sup>104</sup>	Platinic chloride	Spherical crystals of radiating branches
	Gold chloride and hydrochloric acid	Irregular radiating branches
Quinidine <sup>105</sup>	Potassium iodide	Small, triangular crystals in great numbers, best in 1:1000 dilution; crystals soluble in excess reagent
Quinine <sup>106</sup>	Disodium phosphate	Silvery, sheaflike crystals
Scopolamine <sup>107</sup> (Hyoscyne)	Gold chloride	Clusters of pale yellow, transparent blades, with coarse saw-tooth edges form immediately on shaking the slide to stir the solutions. Crystals grow to large size in 1:200 solution

*Characteristics of Microchemical Tests for Alkaloids—Continued*

ALKALOID	REAGENT	DESCRIPTION OF CRYSTALS
Sparteine <sup>107</sup>	Gold chloride	Large numbers of blade-like crystals varying in size according to concentration
Strychnine <sup>108</sup>	Platinic chloride	Crystals form immediately in clusters and singly in small, wedge-shaped needles, which move about the field
	Marmé's	Silvery masses, slowly forming rosettes
Theobromine <sup>109</sup>	Kraut's (freshly prepared)	In hydrochloric acid (1+3). Tufts of brown radiating needles form readily in 1:200 soln.
Theophylline <sup>109</sup>	Ammoniacal silver nitrate	Gelatinous at first; dense spheres of dark radiating needles 1:200. Crystals form more readily if prepared in test tube and then transferred to a glass slide.
Yohimbine <sup>110</sup>	Sodium carbonate	In 1:1000 soln heated to 50°. Fine needles in sheaf-like bundles and in rosettes.

## MICROCHEMICAL TESTS FOR SYNTHETICS—TENTATIVE

## 180

## REAGENTS

- (a) *Phosphotungstic acid soln.*—Dissolve 5 g of  $P_2O_5$  24  $WO_3 \cdot x H_2O$  in 100 cc of  $H_2O$ .
- (b) *Bromide-bromate soln.*—Dissolve 0.3 g of  $KBrO_3$  and 5 g of  $KBr$  in  $H_2O$  and dilute to 100 cc.
- (c) *Nitric acid soln.*—Mix one volume of  $HNO_3$  with one volume of  $H_2O$ .
- (d) *Wagner's.*—Dissolve 1 g of  $I$  and 5 g of  $KI$  in 5 cc of  $H_2O$  and dilute to 100 cc.
- (e) *Mercuric chloride.*—Prepare as directed under 176(e).
- (f) *Marmé's.*—Prepare as directed under 176(i).
- (g) *Potassium ferrocyanide.*—Dissolve 5 g of  $K_4Fe(CN)_6 \cdot 3H_2O$  in 100 cc of  $H_2O$ .
- (h) *Magnesia mixture.*—Dissolve 55 g of  $MgCl_2 \cdot 6H_2O$  and 140 g of  $NH_4Cl$  in  $H_2O$ . Add 130.5 cc of  $NH_4OH$  and  $H_2O$  to make 1 liter.
- (i) *Gold chloride.*—Dissolve 1 g of reagent gold chloride in 20 cc of  $H_2O$ .
- (j) *Hydrochloric acid.*—1%.
- (k) *Silicotungstic acid soln.*—Dissolve 5 g of  $4H_2O \cdot SiO_2 \cdot 12WO_3 \cdot 22H_2O$  in 100 cc of approximately 6  $N H_2SO_4$ .
- (l) *Potassium thiocyanate.*—Dissolve 5 g of  $KSCN$  in 100 cc of  $H_2O$ .
- (m) *Kraut's.*—Dissolve 8 g of  $Bi(NO_3)_3 \cdot 5H_2O$  in 20 cc of  $HNO_3$ , sp. gr. 1.18. Dissolve 27.2 g of  $KI$  in 50 cc of  $H_2O$ . Mix the solns and dilute to 100 cc.

## 181

## PREPARATION OF SAMPLE

Separate the compound in pure form by the use of suitable solvents. Prepare a soln of 1:100 to 1:1000 concentration with the aid of acid, alkali, or  $H_2O$  as specified for the individual synthetics.

*Controls.*—For comparison, prepare a soln of the pure synthetic in the concentration specified for each.

## 182

## IDENTIFICATION

To a drop of a soln of the compound on a clear glass slide, add a drop of the specified reagent, and without stirring or covering examine under the microscope. A magnification of 100–150 is suitable.

SYNTHETIC	SOLUTION	CONCENTRATION OF SYNTHETIC	REAGENT	DESCRIPTION OF TESTS AND CRYSTALS
Acetanilid <sup>11</sup>	10% HCl	1:100	Phosphotungstic acid	Rosettes of prisms
	10% HCl	1:100	Bromide-bromate	Small prisms
Acetphenetidin <sup>11</sup>	About 1 mg of the powdered material		Nitric acid	After adding a drop of nitric acid let stand for a few seconds, then add a drop of H <sub>2</sub> O. Bright yellow, curving, branched crystals
	10% HCl	Saturated soln	Wagner's	Large, irregular plates
Aminopyrine <sup>12</sup>	H <sub>2</sub> O	1:100	Mercuric chloride	Long, slender radiating crystals, often curved
			Marmé's reagent	Groups of spiny branches
Antipyrine <sup>13</sup>	H <sub>2</sub> O	1:100	Potassium ferrocyanide	Acicular and prismatic crystals form after adding a drop of 1% HCl
Benzocaine <sup>14</sup>	HCl. Avoid excess acid	1:100	Potassium ferrocyanide	Colorless, irregular plates and rods
Cinchophen <sup>15</sup>	N NaOH. Add H <sub>2</sub> O, and make slightly acid with HCl	1:1000	Gold chloride	Dark clusters of needles. Few short, rhombic crystals.
Dinitrophenol <sup>15</sup>	Small quantity of 0.1 N NaOH	1:100	Hydrochloric acid	Plates with four branches. In more dilute soln single, rectangular plates.
Methenamine <sup>16</sup>	H <sub>2</sub> O	1:500	Silicotungstic acid	Thin, transparent, rectangular crystals
Neocinchophen <sup>17</sup>	10% HCl	Saturated soln	Potassium thiocyanate	Rosettes of needles. (Gentle agitation by tipping the slide back and forth hastens crystallization)
	10% HCl	Saturated soln	Platinum chloride	Needles in clusters
Oxyquinoline <sup>18</sup> sulfate (Chinosol)	Dissolve the salt in H <sub>2</sub> O. If free base, dissolve in HCl, avoiding excess	1:500	Magnesia mixture	Small, elliptical grains. Few burr-shaped crystals on standing
Pyridium <sup>18</sup>	Dissolve the salt in H <sub>2</sub> O. If free base, dissolve in HCl, avoiding excess	1:1000	Potassium thiocyanate	Small red-brown dense sheaves
Triethanolamine <sup>19</sup>	H <sub>2</sub> O	1:100	Kraut's	Oily globules changing to large red hexagonal plates and prismatic crystals

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- <sup>7</sup> Ibid., 162, p. 193.
- <sup>8</sup> J. Ind. Eng. Chem., 7, 681 (1915).
- <sup>9</sup> J. Assoc. Official Agr. Chem., 5, 582 (1922).
- <sup>10</sup> Ibid., 583.
- <sup>11</sup> Ibid., 9, 49 (1926).
- <sup>12</sup> Ibid., 50.
- <sup>13</sup> J. Ind. Eng. Chem., 7, 519 (1915).
- <sup>14</sup> J. Assoc. Official Agr. Chem., 12, 54 (1929).
- <sup>15</sup> Ibid., 14, 358 (1931).
- <sup>16</sup> Methods of Analysis, A.O.A.C., 1925, p. 390; J. Assoc. Official Agr. Chem., 13, 65 (1930).
- <sup>17</sup> J. Assoc. Official Agr. Chem., 8, 44 (1924); Methods of Analysis, A.O.A.C. 1925, p. 390.
- <sup>18</sup> J. Assoc. Official Agr. Chem., 12, 291 (1929).
- <sup>19</sup> Ibid., 14, 327 (1931).
- <sup>20</sup> Ibid., 13, 329 (1930).
- <sup>21</sup> J. Ind. Eng. Chem., 10, 606 (1918); J. Assoc. Official Agr. Chem., 3, 374 (1920).
- <sup>22</sup> J. Assoc. Official Agr. Chem., 7, 20 (1923).
- <sup>23</sup> Ibid., 9, 52 (1926).
- <sup>24</sup> Landolt, The Optical Rotating Power of Organic Substances, 2nd ed., p. 498 (1902).
- <sup>25</sup> J. Ind. Eng. Chem., 11, 756 (1919).
- <sup>26</sup> Ibid., 14, 24 (1922).
- <sup>27</sup> J. Assoc. Official Agr. Chem., 9, 319 (1926); 10, 47 (1927).
- <sup>28</sup> Ibid., 15, 81 (1932).
- <sup>29</sup> Ibid., 5, 150 (1921).
- <sup>30</sup> Ibid., 10, 49 (1927).
- <sup>31</sup> Ibid., 5, 150 (1921).
- <sup>32</sup> Ibid., 16, 84 (1933).
- <sup>33</sup> Ibid., 18, 89 (1935).
- <sup>34</sup> Ibid., 15, 407 (1932).
- <sup>35</sup> J. Assoc. Official Agr. Chem., 7, 14, (1923).
- <sup>36</sup> J. Ind. Eng. Chem., 12, 766 (1920); J. Assoc. Official Agr. Chem., 8, 541 (1925).
- <sup>37</sup> Ibid., 13, 47 (1930).
- <sup>38</sup> Ibid., 12, 300 (1929).
- <sup>39</sup> Ibid., 54.
- <sup>40</sup> Ibid., 15, 81 (1932).
- <sup>41</sup> Ibid., 7, 29 (1923); 8, 40 (1924).
- <sup>42</sup> Ibid., 11, 51 (1928).
- <sup>43</sup> Ibid., 5, 163, 589 (1921-2).
- <sup>44</sup> Methods of Analysis A.O.A.C., 1925, 404; J. Assoc. Official Agr. Chem., 17, 79 (1934); 19, 106 (1936).
- <sup>45</sup> Methods of Analysis A.O.A.C., 1925, 404.
- <sup>46</sup> J. Assoc. Official Agr. Chem., 9, 53 (1926); 19, 105 (1936).
- <sup>47</sup> Ibid., 11, 49 (1928).
- <sup>48</sup> Ibid., 50.
- <sup>49</sup> U. S. Dept. Agr. Bur. Chem. Circ. 94, p. 4.
- <sup>50</sup> J. Assoc. Official Agr. Chem., 12, 49 (1929).
- <sup>51</sup> Ibid., 14, 360 (1931); Am. J. Pharm., 101, 654 (1929).
- <sup>52</sup> J. Assoc. Official Agr. Chem., 12, 50 (1929).
- <sup>53</sup> Ibid., 17, 78 (1934).
- <sup>54</sup> Ibid., 8, 48, 510 (1925); 9, 51 (1926); 19, 106 (1936).
- <sup>55</sup> Ibid., 19, 106 (1936).
- <sup>56</sup> Ibid., 15, 402 (1932); 18, 84 (1935).

- <sup>57</sup> Ibid., 16, 81 (1933).
- <sup>58</sup> Ibid., 18, 88 (1935).
- <sup>59</sup> Ibid., 16, 357 (1933).
- <sup>60</sup> Ibid., 17, 446 (1934); 18, 88 (1935).
- <sup>61</sup> Ibid., 19, 104 (1936).
- <sup>62</sup> Ibid., 14, 321 (1931).
- <sup>63</sup> Ibid., 18, 87 (1935).
- <sup>64</sup> Ibid., 19, 104 (1936).
- <sup>65</sup> Ibid., 16, 83 (1933).
- <sup>66</sup> Ibid., 15, 83 (1932).
- <sup>67</sup> Ibid., 19, 105 (1936).
- <sup>68</sup> Ibid., 10, 44 (1927).
- <sup>69</sup> Ibid., 11, 48 (1928).
- <sup>70</sup> Ibid., 49.
- <sup>71</sup> Ibid., 15, 84 (1932).
- <sup>72</sup> Ibid., 17, 75 (1934).
- <sup>73</sup> Ibid., 18, 87 (1935).
- <sup>74</sup> Ibid., 15, 83 (1932).
- <sup>75</sup> Ibid., 14, 377 (1931).
- <sup>76</sup> Ibid., 15, 85 (1932).
- <sup>77</sup> Ibid., 11, 51 (1928); 12, 52 (1929).
- <sup>78</sup> Ibid., 14, 312 (1931).
- <sup>79</sup> Ibid., 12, 52 (1929).
- <sup>80</sup> Ibid., 17, 75 (1934).
- <sup>81</sup> Ibid., 76.
- <sup>82</sup> Ibid., 18, 85 (1935).
- <sup>83</sup> Ibid., 89.
- <sup>84</sup> Ibid., 16, 83, 292 (1934).
- <sup>85</sup> Ibid., 11, 53 (1928).
- <sup>86</sup> Ibid., 8, 551 (1925); 9, 54 (1926).
- <sup>87</sup> Ibid., 55; 10, 46 (1927).
- <sup>88</sup> Ibid., 13, 334 (1930); 14, 53 (1931).
- <sup>89</sup> Ibid., 11, 53 (1928).
- <sup>90</sup> Methods of Analysis, A.O.A.C., 1930, p. 487; J. Assoc. Official Agr. Chem., 14, 51 (1931).
- <sup>91</sup> J. Assoc. Official Agr. Chem., 15, 81 (1932).
- <sup>92</sup> Ibid., 11, 354 (1928); 12, 284 (1929); 14, 51, 317 (1931).
- <sup>93</sup> Ibid., 13, 46 (1930).
- <sup>94</sup> Ibid., 12, 53 (1929).
- <sup>95</sup> Ibid., 10, 372 (1927); 11, 52 (1928).
- <sup>96</sup> Ibid., 14, 317 (1931).
- <sup>97</sup> J. Ind. Eng. Chem., 4, 508 (1912); J. Assoc. Official Agr. Chem., 5, 154 (1921); 10, 372 (1927); 11, 52, 354 (1928).
- <sup>98</sup> J. Assoc. Official Agr. Chem., 18, 85 (1935).
- <sup>99</sup> Ibid., 18, 86 (1935).
- <sup>100</sup> Ibid., 10, 372 (1927); 11, 52, 354 (1928).
- <sup>101</sup> Ibid., 16, 82 (1933).
- <sup>102</sup> Ibid., 17, 76 (1934).
- <sup>103</sup> Ibid., 11, 354 (1928).
- <sup>104</sup> Ibid., 17, 76 (1934).
- <sup>105</sup> Ibid., 12, 53 (1929).
- <sup>106</sup> Ibid., 18, 86 (1935).
- <sup>107</sup> Ibid., 16, 82 (1934).
- <sup>108</sup> Ibid., 11, 52, 354 (1928).
- <sup>109</sup> Ibid., 19, 103 (1936).
- <sup>110</sup> Ibid., 15, 81 (1932).
- <sup>111</sup> Ibid., 19, 103 (1936).
- <sup>112</sup> Ibid., 18, 86 (1935).
- <sup>113</sup> Ibid., 17, 77 (1934).
- <sup>114</sup> Ibid., 16, 84 (1933).
- <sup>115</sup> Ibid., 18, 86 (1935).
- <sup>116</sup> Ibid., 17, 77 (1934).
- <sup>117</sup> Ibid., 19, 104 (1936).
- <sup>118</sup> Ibid., 16, 84 (1933).
- <sup>119</sup> Ibid., 17, 77 (1934).

## XL. BACTERIOLOGICAL METHODS\*

## XLI. MICROCHEMICAL METHODS\*

\* See note at bottom of p. xvii.

Various strength solutions of the common acids, alkalies, and alcohol.<sup>1</sup> 2

(a) *Hydrochloric Acid Solutions*: Specification requires not less than 35% HCl by weight. Sp. gr. = 1.1778 at 15°. Mix with water and make up to 1 liter.

HCl STRENGTH DESIRED GRAMS PER LITER	HYDROCHLORIC ACID REQUIRED		
	g	cc	
5	14.29	12.13	Normal solution
10	28.57	24.26	
15	42.85	36.39	
20	57.14	48.52	
36.46	104.17	88.45	
50	142.86	121.29	
100	285.71	242.58	
150	428.57	363.88	
200	571.43	485.17	Constant boiling Sp. gr. 1.125
222.6	636.00	539.99	
278.4	795.43	675.35	
300	857.14	727.75	

(b) *Sulfuric Acid Solutions*: Specification requires not less than 94% H<sub>2</sub>SO<sub>4</sub> by weight. Sp. gr. = 1.835 at 15°. Pour acid into excess of water and make up to 1 liter.

H <sub>2</sub> SO <sub>4</sub> STRENGTH DESIRED GRAMS PER LITER	SULFURIC ACID REQUIRED		
	g	cc	
5	5.32	3.0	For crude fiber
12.5	13.29	7.2	
20	21.28	11.6	
30	31.91	17.4	
40	42.55	23.2	Normal solution
49	52.13	28.4	
100	106.38	58.0	
150	159.57	87.0	
250	265.96	144.9	
300	319.15	173.9	
400	425.53	231.9	

(c) *Nitric Acid Solutions*: Specification requires not less than 68% HNO<sub>3</sub> by weight. Sp. gr. = 1.4146 at 15°. 1 cc of concentrated HNO<sub>3</sub> contains approximately 0.96 g of HNO<sub>3</sub>. Mix with water and make up to 1 liter.

HNO <sub>3</sub> STRENGTH DESIRED GRAMS PER LITER	NITRIC ACID REQUIRED	
	g	cc
5	7.35	5.2
10	14.71	10.4
20	29.41	20.8
30	44.12	31.2
40	58.82	41.6
50	73.53	52.0
63	92.65	65.5
70	102.94	72.8
100	147.06	104.0
150	220.59	156.0
200	294.12	207.9
300	441.18	312.9

<sup>1</sup> Prepared by G. C. Spencer and H. J. Fisher.

2 *Various strength solutions of common acids, alkalies, and alcohol.*—Concluded.

(d) *Ammonia Solutions:* Specification requires not less than 27%  $\text{NH}_3$  by weight. Sp. gr. = 0.9. Mix and make to 1 liter.

NH <sub>3</sub> STRENGTH DESIRED GRAMS PER LITER	REAGENT AMMONIA REQUIRED	
	g	cc
5	18.52	20.6
10	37.04	41.1
15	55.55	61.7
20	74.07	82.3
25	92.59	102.9
50	185.18	205.8
75	277.77	308.6
100	370.37	411.5
150	555.55	617.3
200	740.74	823.0

(e) *Sodium Hydroxide Solutions:* Specification requires 95% NaOH in sticks of caustic soda. Dissolve and dilute to 1 liter.

NaOH STRENGTH DESIRED GRAMS PER LITER	SODIUM HYDROXIDE REQUIRED	
	g	
12.5	13.16	For crude fiber  Normal solution
30	31.58	
40	42.11	
50	52.63	
75	78.95	
100	105.26	
150	157.89	
200	210.53	
250	263.16	
300	315.79	

(f) *Alcoholic Solutions:* Specification requires 95%  $\text{C}_2\text{H}_5\text{OH}$  by volume. Sp. gr. = 0.810 at 25°. Mix and make to 1 liter.

ALCOHOL STRENGTH DESIRED CC PER LITER	ALCOHOL REQUIRED	
	cc	g
50	52.6	42.63
100	105.3	85.26
150	157.9	127.89
200	210.5	170.52
250	263.2	213.16
300	315.9	255.78
400	421.1	341.04
500	526.3	426.32 (proof)
700	736.8	596.84

<sup>1</sup> Alcohol of any desired strength may be obtained by taking the number of cc of 95% alcohol equivalent to the desired strength and making the solution up to 95 cc. For example.—To obtain a solution of 70° alcohol, take 70 cc of 95% alcohol and dilute to 95 cc.

*Degrees Brix, specific gravity, and degrees Baumé of sugar solutions<sup>1</sup>*  
(Plato's Table<sup>2</sup>).

3

DEGREES BRIX OR PER CENT BY WEIGHT OF SUCROSE	SPECIFIC GRAVITY AT 20/20°C.	SPECIFIC GRAVITY AT 20/4°C.	DEGREES BAUMÉ (MODULUS 145)	DEGREES BRIX OR PER CENT BY WEIGHT OF SUCROSE	SPECIFIC GRAVITY AT 20/20°C.	SPECIFIC GRAVITY AT 20/4°C.	DEGREES BAUMÉ (MODULUS 145)
0.0	1.00000	0.998234	0.00	9.0	1.03586	1.034029	5.02
0.2	1.00078	0.999010	0.11	9.2	1.03668	1.034850	5.13
0.4	1.00155	0.999786	0.22	9.4	1.03750	1.035671	5.24
0.6	1.00233	1.000563	0.34	9.6	1.03833	1.036494	5.35
0.8	1.00311	1.001342	0.45	9.8	1.03915	1.037318	5.46
1.0	1.00389	1.002120	0.56	10.0	1.03998	1.038143	5.57
1.2	1.00467	1.002897	0.67	10.2	1.04081	1.038970	5.68
1.4	1.00545	1.003675	0.79	10.4	1.04164	1.039797	5.80
1.6	1.00623	1.004453	0.90	10.6	1.04247	1.040626	5.91
1.8	1.00701	1.005234	1.01	10.8	1.04330	1.041456	6.02
2.0	1.00779	1.006015	1.12	11.0	1.04413	1.042288	6.13
2.2	1.00858	1.006796	1.23	11.2	1.04497	1.043121	6.24
2.4	1.00936	1.007580	1.34	11.4	1.04580	1.043954	6.35
2.6	1.01015	1.008363	1.46	11.6	1.04664	1.044788	6.46
2.8	1.01093	1.009148	1.57	11.8	1.04747	1.045625	6.57
3.0	1.01172	1.009934	1.68	12.0	1.04831	1.046462	6.68
3.2	1.01251	1.010721	1.79	12.2	1.04915	1.047300	6.79
3.4	1.01330	1.011510	1.90	12.4	1.04999	1.048140	6.90
3.6	1.01409	1.012298	2.02	12.6	1.05084	1.048980	7.02
3.8	1.01488	1.013089	2.13	12.8	1.05168	1.049822	7.13
4.0	1.01567	1.013881	2.24	13.0	1.05252	1.050665	7.24
4.2	1.01647	1.014673	2.35	13.2	1.05337	1.051510	7.35
4.4	1.01726	1.015467	2.46	13.4	1.05422	1.052356	7.46
4.6	1.01806	1.016261	2.57	13.6	1.05506	1.053202	7.57
4.8	1.01886	1.017058	2.68	13.8	1.05591	1.054050	7.68
5.0	1.01965	1.017854	2.79	14.0	1.05677	1.054900	7.79
5.2	1.02045	1.018652	2.91	14.2	1.05762	1.055751	7.90
5.4	1.02125	1.019451	3.02	14.4	1.05847	1.056602	8.01
5.6	1.02206	1.020251	3.13	14.6	1.05933	1.057455	8.12
5.8	1.02286	1.021053	3.24	14.8	1.06018	1.058310	8.23
6.0	1.02366	1.021855	3.35	15.0	1.06104	1.059165	8.34
6.2	1.02447	1.022659	3.46	15.2	1.06190	1.060022	8.45
6.4	1.02527	1.023463	3.57	15.4	1.06276	1.060880	8.56
6.6	1.02608	1.024270	3.69	15.6	1.06362	1.061738	8.67
6.8	1.02689	1.025077	3.80	15.8	1.06448	1.062598	8.78
7.0	1.02770	1.025885	3.91	16.0	1.06534	1.063460	8.89
7.2	1.02851	1.026694	4.02	16.2	1.06621	1.064324	9.00
7.4	1.02932	1.027504	4.13	16.4	1.06707	1.065188	9.11
7.6	1.03013	1.028316	4.24	16.6	1.06794	1.066054	9.22
7.8	1.03095	1.029128	4.35	16.8	1.06881	1.066921	9.33
8.0	1.03176	1.029942	4.46	17.0	1.06968	1.067789	9.45
8.2	1.03258	1.030757	4.58	17.2	1.07055	1.068658	9.56
8.4	1.03340	1.031573	4.69	17.4	1.07142	1.069529	9.67
8.6	1.03422	1.032391	4.80	17.6	1.07229	1.070400	9.78
8.8	1.03504	1.033209	4.91	17.8	1.07317	1.071273	9.89

<sup>1</sup> Bur. Standards Circ. 44, 1918, p. 151.<sup>2</sup> Based upon figures prepared by the Kaiserliche Normal-Eichungs-Kommission and accepted by the International Commission for Unifying Methods of Sugar Analysis.



## 3 Degrees Briz, specific gravity, and degrees Baumé of sugar solutions.—Continued.

DEGREES BRIZ OR PER CENT BY WEIGHT OF SUCROSE	SPECIFIC GRAVITY AT 20/20°C.	SPECIFIC GRAVITY AT 20/4°C.	DEGREES BAUMÉ (MODULUS 145)	DEGREES BRIZ OR PER CENT BY WEIGHT OF SUCROSE	SPECIFIC GRAVITY AT 20/20°C.	SPECIFIC GRAVITY AT 20/4°C.	DEGREES BAUMÉ (MODULUS 145)
18.0	1.07404	1.072147	10.00	27.0	1.11480	1.112828	14.93
18.2	1.07492	1.073023	10.11	27.2	1.11573	1.113763	15.04
18.4	1.07580	1.073900	10.22	27.4	1.11667	1.114697	15.15
18.6	1.07668	1.074777	10.33	27.6	1.11761	1.115635	15.26
18.8	1.07756	1.075657	10.44	27.8	1.11855	1.116572	15.37
19.0	1.07844	1.076537	10.55	28.0	1.11949	1.117512	15.48
19.2	1.07932	1.077419	10.66	28.2	1.12043	1.118453	15.59
19.4	1.08021	1.078302	10.77	28.4	1.12138	1.119395	15.69
19.6	1.08110	1.079187	10.88	28.6	1.12232	1.120339	15.80
19.8	1.08198	1.080072	10.99	28.8	1.12327	1.121284	15.91
20.0	1.08287	1.080959	11.10	29.0	1.12422	1.122231	16.02
20.2	1.08376	1.081848	11.21	29.2	1.12517	1.123179	16.13
20.4	1.08465	1.082737	11.32	29.4	1.12612	1.124128	16.24
20.6	1.08554	1.083628	11.43	29.6	1.12707	1.125079	16.35
20.8	1.08644	1.084520	11.54	29.8	1.12802	1.126030	16.46
21.0	1.08733	1.085414	11.65	30.0	1.12898	1.126984	16.57
21.2	1.08823	1.086309	11.76	30.2	1.12993	1.127939	16.67
21.4	1.08913	1.087205	11.87	30.4	1.13089	1.128896	16.78
21.6	1.09003	1.088101	11.98	30.6	1.13185	1.129853	16.89
21.8	1.09093	1.089000	12.09	30.8	1.13281	1.130812	17.00
22.0	1.09183	1.089900	12.20	31.0	1.13378	1.131773	17.11
22.2	1.09273	1.090802	12.31	31.2	1.13474	1.132735	17.22
22.4	1.09364	1.091704	12.42	31.4	1.13570	1.133698	17.33
22.6	1.09454	1.092607	12.52	31.6	1.13667	1.134663	17.43
22.8	1.09545	1.093513	12.63	31.8	1.13764	1.135628	17.54
23.0	1.09636	1.094420	12.74	32.0	1.13861	1.136596	17.65
23.2	1.09727	1.095328	12.85	32.2	1.13958	1.137565	17.76
23.4	1.09818	1.096236	12.96	32.4	1.14055	1.138534	17.87
23.6	1.09909	1.097147	13.07	32.6	1.14152	1.139506	17.98
23.8	1.10000	1.098058	13.18	32.8	1.14250	1.140479	18.08
24.0	1.10092	1.098971	13.29	33.0	1.14347	1.141453	18.19
24.2	1.10183	1.099886	13.40	33.2	1.14445	1.142429	18.30
24.4	1.10275	1.100802	13.51	33.4	1.14543	1.143405	18.41
24.6	1.10367	1.101718	13.62	33.6	1.14641	1.144384	18.52
24.8	1.10459	1.102637	13.73	33.8	1.14739	1.145363	18.63
25.0	1.10551	1.103557	13.84	34.0	1.14837	1.146345	18.73
25.2	1.10643	1.104478	13.95	34.2	1.14936	1.147328	18.84
25.4	1.10736	1.105400	14.06	34.4	1.15034	1.148313	18.95
25.6	1.10828	1.106324	14.17	34.6	1.15133	1.149298	19.06
25.8	1.10921	1.107248	14.28	34.8	1.15232	1.150286	19.17
26.0	1.11014	1.108175	14.39	35.0	1.15331	1.151275	19.28
26.2	1.11106	1.109103	14.49	35.2	1.15430	1.152265	19.38
26.4	1.11200	1.110033	14.60	35.4	1.15530	1.153256	19.49
26.6	1.11293	1.110963	14.71	35.6	1.15629	1.154249	19.60
26.8	1.11386	1.111895	14.82	35.8	1.15729	1.155242	19.71

*Degrees Brix, specific gravity, and degrees Baumé of sugar solutions.—Continued. 3*

DEGREES BRIX OR PER CENT BY WEIGHT OF SUCROSE	SPECIFIC GRAVITY AT 20/20°C.	SPECIFIC GRAVITY AT 20/4°C.	DEGREES BAUMÉ (MODULUS 145)	DEGREES BRIX OR PER CENT BY WEIGHT OF SUCROSE	SPECIFIC GRAVITY AT 20/20°C.	SPECIFIC GRAVITY AT 20/4°C.	DEGREES BAUMÉ (MODULUS 145)
36.0	1.15828	1.156238	19.81	45.0	1.20467	1.202540	24.63
36.2	1.15928	1.157235	19.92	45.2	1.20573	1.203603	24.74
36.4	1.16028	1.158233	20.03	45.4	1.20680	1.204668	24.85
36.6	1.16128	1.159233	20.14	45.6	1.20787	1.205733	24.95
36.8	1.16228	1.160233	20.25	45.8	1.20894	1.206801	25.06
37.0	1.16329	1.161236	20.35	46.0	1.21001	1.207870	25.17
37.2	1.16430	1.162240	20.46	46.2	1.21108	1.208940	25.27
37.4	1.16530	1.163245	20.57	46.4	1.21215	1.210013	25.38
37.6	1.16631	1.164252	20.68	46.6	1.21323	1.211086	25.48
37.8	1.16732	1.165259	20.78	46.8	1.21431	1.212162	25.59
38.0	1.16833	1.166269	20.89	47.0	1.21538	1.213238	25.70
38.2	1.16934	1.167281	21.00	47.2	1.21646	1.214317	25.80
38.4	1.17036	1.168293	21.11	47.4	1.21755	1.215395	25.91
38.6	1.17138	1.169307	21.21	47.6	1.21863	1.216476	26.01
38.8	1.17239	1.170322	21.32	47.8	1.21971	1.217559	26.12
39.0	1.17341	1.171340	21.43	48.0	1.22080	1.218643	26.23
39.2	1.17443	1.172359	21.54	48.2	1.22189	1.219729	26.33
39.4	1.17545	1.173379	21.64	48.4	1.22298	1.220815	26.44
39.6	1.17648	1.174400	21.75	48.6	1.22406	1.221904	26.54
39.8	1.17750	1.175423	21.86	48.8	1.22516	1.222995	26.65
40.0	1.17853	1.176447	21.97	49.0	1.22625	1.224086	26.75
40.2	1.17956	1.177473	22.07	49.2	1.22735	1.225180	26.86
40.4	1.18058	1.178501	22.18	49.4	1.22844	1.226274	26.96
40.6	1.18162	1.179527	22.29	49.6	1.22954	1.227371	27.07
40.8	1.18265	1.180560	22.39	49.8	1.23064	1.228469	27.18
41.0	1.18368	1.181592	22.50	50.0	1.23174	1.229567	27.28
41.2	1.18472	1.182625	22.61	50.2	1.23284	1.230668	27.39
41.4	1.18575	1.183660	22.72	50.4	1.23395	1.231770	27.49
41.6	1.18679	1.184696	22.82	50.6	1.23506	1.232874	27.60
41.8	1.18783	1.185734	22.93	50.8	1.23616	1.233979	27.70
42.0	1.18887	1.186773	23.04	51.0	1.23727	1.235085	27.81
42.2	1.18992	1.187814	23.14	51.2	1.23838	1.236194	27.91
42.4	1.19096	1.188856	23.25	51.4	1.23949	1.237303	28.02
42.6	1.19201	1.189901	23.36	51.6	1.24060	1.238414	28.12
42.8	1.19305	1.190946	23.46	51.8	1.24172	1.239527	28.23
43.0	1.19410	1.191993	23.57	52.0	1.24284	1.240641	28.33
43.2	1.19515	1.193041	23.68	52.2	1.24395	1.241757	28.44
43.4	1.19620	1.194090	23.78	52.4	1.24507	1.242873	28.54
43.6	1.19726	1.195141	23.89	52.6	1.24619	1.243992	28.65
43.8	1.19831	1.196193	24.00	52.8	1.24731	1.245113	28.75
44.0	1.19936	1.197247	24.10	53.0	1.24844	1.246234	28.86
44.2	1.20042	1.198303	24.21	53.2	1.24956	1.247358	28.96
44.4	1.20148	1.199360	24.32	53.4	1.25069	1.248482	29.06
44.6	1.20254	1.200420	24.42	53.6	1.25182	1.249609	29.17
44.8	1.20360	1.201480	24.53	53.8	1.25295	1.250737	29.27

## 3 Degrees Brix, specific gravity, and degrees Baumé of sugar solutions.—Continued.

DEGREES BRIX OR PER CENT BY WEIGHT OF SUCROSE	SPECIFIC GRAVITY AT 20/20°C.	SPECIFIC GRAVITY AT 20/4°C.	DEGREES BAUMÉ (MODULUS 145)	DEGREES BRIX OR PER CENT BY WEIGHT OF SUCROSE	SPECIFIC GRAVITY AT 20/20°C.	SPECIFIC GRAVITY AT 20/4°C.	DEGREES BAUMÉ (MODULUS 145)
54.0	1.25408	1.251866	29.38	63.0	1.30657	1.304267	34.02
54.2	1.25521	1.252997	29.48	63.2	1.30778	1.305467	34.12
54.4	1.25635	1.254129	29.59	63.4	1.30898	1.306669	34.23
54.6	1.25748	1.255264	29.69	63.6	1.31019	1.307872	34.33
54.8	1.25862	1.256400	29.80	63.8	1.31139	1.309077	34.43
55.0	1.25976	1.257535	29.90	64.0	1.31260	1.310282	34.53
55.2	1.26090	1.258674	30.00	64.2	1.31381	1.311489	34.63
55.4	1.26204	1.259815	30.11	64.4	1.31502	1.312699	34.74
55.6	1.26319	1.260955	30.21	64.6	1.31623	1.313909	34.84
55.8	1.26433	1.262099	30.32	64.8	1.31745	1.315121	34.94
56.0	1.26548	1.263243	30.42	65.0	1.31866	1.316334	35.04
56.2	1.26663	1.264390	30.52	65.2	1.31988	1.317549	35.14
56.4	1.26778	1.265537	30.63	65.4	1.32110	1.318766	35.24
56.6	1.26893	1.266686	30.73	65.6	1.32232	1.319983	35.34
56.8	1.27008	1.267837	30.83	65.8	1.32354	1.321203	35.45
57.0	1.27123	1.268980	30.94	66.0	1.32476	1.322425	35.55
57.2	1.27239	1.270143	31.04	66.2	1.32599	1.323648	35.65
57.4	1.27355	1.271299	31.15	66.4	1.32722	1.324872	35.75
57.6	1.27471	1.272455	31.25	66.6	1.32844	1.326097	35.85
57.8	1.27587	1.273614	31.35	66.8	1.32967	1.327325	35.95
58.0	1.27703	1.274774	31.46	67.0	1.33090	1.328554	36.05
58.2	1.27819	1.275936	31.56	67.2	1.33214	1.329785	36.15
58.4	1.27936	1.277098	31.66	67.4	1.33337	1.331017	36.25
58.6	1.28052	1.278262	31.76	67.6	1.33460	1.332250	36.35
58.8	1.28169	1.279428	31.87	67.8	1.33584	1.333485	36.45
59.0	1.28286	1.280595	31.97	68.0	1.33708	1.334722	36.55
59.2	1.28404	1.281764	32.07	68.2	1.33832	1.335961	36.66
59.4	1.28520	1.282935	32.18	68.4	1.33957	1.337200	36.76
59.6	1.28638	1.284107	32.28	68.6	1.34081	1.338441	36.86
59.8	1.28755	1.285281	32.38	68.8	1.34205	1.339684	36.96
60.0	1.28873	1.286456	32.49	69.0	1.34330	1.340928	37.06
60.2	1.28991	1.287633	32.59	69.2	1.34455	1.342174	37.16
60.4	1.29109	1.288811	32.69	69.4	1.34580	1.343421	37.26
60.6	1.29227	1.289991	32.79	69.6	1.34705	1.344671	37.36
60.8	1.29346	1.291172	32.90	69.8	1.34830	1.345922	37.46
61.0	1.29464	1.292354	33.00	70.0	1.34956	1.347174	37.56
61.2	1.29583	1.293539	33.10	70.2	1.35081	1.348427	37.66
61.4	1.29701	1.294725	33.20	70.4	1.35207	1.349682	37.76
61.6	1.29820	1.295911	33.31	70.6	1.35333	1.350939	37.86
61.8	1.29940	1.297100	33.41	70.8	1.35459	1.352197	37.96
62.0	1.30059	1.298291	33.51	71.0	1.35585	1.353456	38.06
62.2	1.30178	1.299483	33.61	71.2	1.35711	1.354717	38.16
62.4	1.30298	1.300677	33.72	71.4	1.35838	1.355980	38.26
62.6	1.30418	1.301871	33.82	71.6	1.35964	1.357245	38.35
62.8	1.30537	1.303068	33.92	71.8	1.36091	1.358511	38.45

*Degrees Brix, specific gravity, and degrees Baumé of sugar solutions.—Continued. 3*

DEGREES BRIX OR PER CENT BY WEIGHT OF SUCROSE	SPECIFIC GRAVITY AT 20/20°C.	SPECIFIC GRAVITY AT 20/4°C.	DEGREES BAUMÉ (MODULUS 145)	DEGREES BRIX OR PER CENT BY WEIGHT OF SUCROSE	SPECIFIC GRAVITY AT 20/20°C.	SPECIFIC GRAVITY AT 20/4°C.	DEGREES BAUMÉ (MODULUS 145)
72.0	1.36218	1.359778	38.55	81.0	1.42088	1.418374	42.95
72.2	1.36346	1.361047	38.65	81.2	1.42222	1.419711	43.05
72.4	1.36473	1.362317	38.75	81.4	1.42356	1.421049	43.14
72.6	1.36600	1.363590	38.85	81.6	1.42490	1.422390	43.24
72.8	1.36728	1.364864	38.95	81.8	1.42625	1.423730	43.33
73.0	1.36856	1.366139	39.05	82.0	1.42759	1.425072	43.43
73.2	1.36983	1.367415	39.15	82.2	1.42894	1.426416	43.53
73.4	1.37111	1.368693	39.25	82.4	1.43029	1.427761	43.62
73.6	1.37240	1.369973	39.35	82.6	1.43164	1.429109	43.72
73.8	1.37368	1.371254	39.44	82.8	1.43298	1.430457	43.81
74.0	1.37496	1.372536	39.54	83.0	1.43434	1.431807	43.91
74.2	1.37625	1.373820	39.64	83.2	1.43569	1.433158	44.00
74.4	1.37754	1.375105	39.74	83.4	1.43705	1.434511	44.10
74.6	1.37883	1.376392	39.84	83.6	1.43841	1.435866	44.19
74.8	1.38012	1.377680	39.94	83.8	1.43976	1.437222	44.29
75.0	1.38141	1.378971	40.03	84.0	1.44112	1.438579	44.38
75.2	1.38270	1.380262	40.13	84.2	1.44249	1.439938	44.48
75.4	1.38400	1.381555	40.23	84.4	1.44385	1.441299	44.57
75.6	1.38530	1.382851	40.33	84.6	1.44521	1.442661	44.67
75.8	1.38660	1.384148	40.43	84.8	1.44658	1.444024	44.76
76.0	1.38790	1.385446	40.53	85.0	1.44794	1.445388	44.86
76.2	1.38920	1.386745	40.62	85.2	1.44931	1.446750	44.95
76.4	1.39050	1.388045	40.72	85.4	1.45068	1.448121	45.05
76.6	1.39180	1.389347	40.82	85.6	1.45205	1.449491	45.14
76.8	1.39311	1.390651	40.92	85.8	1.45343	1.450860	45.24
77.0	1.39442	1.391956	41.01	86.0	1.45480	1.452232	45.33
77.2	1.39573	1.393263	41.11	86.2	1.45618	1.453605	45.42
77.4	1.39704	1.394571	41.21	86.4	1.45755	1.454980	45.52
77.6	1.39835	1.395881	41.31	86.6	1.45893	1.456357	45.61
77.8	1.39966	1.397192	41.40	86.8	1.46031	1.457735	45.71
78.0	1.40098	1.398505	41.50	87.0	1.46170	1.459114	45.80
78.2	1.40230	1.399819	41.60	87.2	1.46308	1.460495	45.89
78.4	1.40361	1.401134	41.70	87.4	1.46446	1.461877	45.99
78.6	1.40493	1.402452	41.79	87.6	1.46585	1.463260	46.08
78.8	1.40625	1.403771	41.89	87.8	1.46724	1.464645	46.17
79.0	1.40758	1.405091	41.99	88.0	1.46862	1.466032	46.27
79.2	1.40890	1.406412	42.08	88.2	1.47002	1.467420	46.36
79.4	1.41023	1.407735	42.18	88.4	1.47141	1.468810	46.45
79.6	1.41155	1.409061	42.28	88.6	1.47280	1.470200	46.55
79.8	1.41288	1.410387	42.37	88.8	1.47420	1.471592	46.64
80.0	1.41421	1.411715	42.47	89.0	1.47559	1.472986	46.73
80.2	1.41554	1.413044	42.57	89.2	1.47699	1.474381	46.83
80.4	1.41688	1.414374	42.66	89.4	1.47839	1.475779	46.92
80.6	1.41821	1.415706	42.76	89.6	1.47979	1.477176	47.01
80.8	1.41955	1.417039	42.85	89.8	1.48119	1.478575	47.11

## 3 Degrees Brix, specific gravity, and degrees Baumé of sugar solutions.—Concluded.

DEGREES BRIX OR PER CENT BY WEIGHT OF SUCROSE	SPECIFIC GRAVITY AT 20/20°C.	SPECIFIC GRAVITY AT 20/4°C.	DEGREES BAUMÉ (MODULUS 145)	DEGREES BRIX OR PER CENT BY WEIGHT OF SUCROSE	SPECIFIC GRAVITY AT 20/20°C.	SPECIFIC GRAVITY AT 20/4°C.	DEGREES BAUMÉ (MODULUS 145)
90.0	1.48259	1.479976	47.20	95.0	1.51814	1.515455	49.49
90.2	1.48400	1.481378	47.29	95.2	1.51958	1.516893	49.58
90.4	1.48540	1.482782	47.38	95.4	1.52102	1.518332	49.67
90.6	1.48681	1.484187	47.48	95.6	1.52246	1.519771	49.76
90.8	1.48822	1.485593	47.57	95.8	1.52390	1.521212	49.85
91.0	1.48963	1.487002	47.66	96.0	1.52535	1.522656	49.94
91.2	1.49104	1.488411	47.75	96.2	1.52680	1.524100	50.03
91.4	1.49246	1.489823	47.84	96.4	1.52824	1.525546	50.12
91.6	1.49387	1.491234	47.94	96.6	1.52969	1.526993	50.21
91.8	1.49529	1.492647	48.03	96.8	1.53114	1.528441	50.30
92.0	1.49671	1.494063	48.12	97.0	1.53260	1.529891	50.39
92.2	1.49812	1.495479	48.21	97.2	1.53405	1.531342	50.48
92.4	1.49954	1.496897	48.30	97.4	1.53551	1.532794	50.57
92.6	1.50097	1.498316	48.40	97.6	1.53696	1.534248	50.66
92.8	1.50239	1.499736	48.49	97.8	1.53842	1.535704	50.75
93.0	1.50381	1.501158	48.58	98.0	1.53988	1.537161	50.84
93.2	1.50524	1.502582	48.67	98.2	1.54134	1.538618	50.93
93.4	1.50667	1.504006	48.76	98.4	1.54280	1.540076	51.02
93.6	1.50810	1.505432	48.85	98.6	1.54426	1.541536	51.10
93.8	1.50952	1.506859	48.94	98.8	1.54573	1.542998	51.19
94.0	1.51096	1.508289	49.03	99.0	1.54719	1.544462	51.28
94.2	1.51239	1.509720	49.12	99.2	1.54866	1.545926	51.37
94.4	1.51382	1.511151	49.22	99.4	1.55013	1.547392	51.46
94.6	1.51526	1.512585	49.31	99.6	1.55160	1.548861	51.55
94.8	1.51670	1.514019	49.40	99.8	1.55307	1.550329	51.64
				100.0	1.55454	1.551800	51.73

*Temperature corrections to readings of saccharimeters (standard at 20°C). 4*

(This table is calculated from the data on thermal expansion of sugar solutions by Plato,<sup>1</sup> and it is assumed that the instrument is of Jena 16<sup>111</sup> glass. The table should be used with caution and only for approximate results when the temperature differs much from the standard temperature or from the temperature of the surrounding air.)

TEMPERATURE IN DEGREES CENTIGRADE	OBSERVED PERCENTAGE OF SUGAR													
	0	5	10	15	20	25	30	35	40	45	50	55	60	70
	Subtract—													
0	0.30	0.49	0.65	0.77	0.89	0.99	1.08	1.16	1.24	1.31	1.37	1.41	1.44	1.49
5	0.36	0.47	0.56	0.65	0.73	0.80	0.86	0.91	0.97	1.01	1.05	1.08	1.10	1.14
10	0.32	0.38	0.43	0.48	0.52	0.57	0.60	0.64	0.67	0.70	0.72	0.74	0.75	0.77
11	0.31	0.35	0.40	0.44	0.48	0.51	0.55	0.58	0.60	0.63	0.65	0.66	0.68	0.70
12	0.29	0.32	0.36	0.40	0.43	0.46	0.50	0.52	0.54	0.56	0.58	0.59	0.60	0.62
13	0.26	0.29	0.32	0.35	0.38	0.41	0.44	0.46	0.48	0.49	0.51	0.52	0.53	0.55
14	0.24	0.26	0.29	0.31	0.34	0.36	0.38	0.40	0.41	0.42	0.44	0.45	0.46	0.47
15	0.20	0.22	0.24	0.26	0.28	0.30	0.32	0.33	0.34	0.36	0.36	0.37	0.38	0.39
16	0.17	0.18	0.20	0.22	0.23	0.25	0.26	0.27	0.28	0.28	0.29	0.30	0.31	0.32
17	0.13	0.14	0.15	0.16	0.18	0.19	0.20	0.20	0.21	0.21	0.22	0.23	0.23	0.24
18	0.09	0.10	0.10	0.11	0.12	0.13	0.13	0.14	0.14	0.14	0.15	0.15	0.15	0.16
19	0.05	0.05	0.05	0.06	0.06	0.06	0.07	0.07	0.07	0.07	0.08	0.08	0.08	0.80
17.5	0.11	0.12	0.12	0.14	0.15	0.16	0.16	0.17	0.17	0.18	0.18	0.19	0.19	0.20
15.56 (60°F.)	0.18	0.20	0.22	0.24	0.26	0.28	0.29	0.30	0.30	0.32	0.33	0.33	0.34	0.34
	Add—													
21	0.04	0.05	0.06	0.06	0.06	0.07	0.07	0.07	0.07	0.08	0.08	0.08	0.08	0.09
22	0.10	0.10	0.11	0.12	0.12	0.13	0.14	0.14	0.15	0.15	0.16	0.16	0.16	0.16
23	0.16	0.16	0.17	0.17	0.19	0.20	0.21	0.21	0.22	0.23	0.24	0.24	0.24	0.24
24	0.21	0.22	0.23	0.24	0.26	0.27	0.28	0.29	0.30	0.31	0.32	0.32	0.32	0.32
25	0.27	0.28	0.30	0.31	0.32	0.34	0.35	0.36	0.38	0.38	0.39	0.39	0.40	0.39
26	0.33	0.34	0.36	0.37	0.40	0.40	0.42	0.44	0.46	0.47	0.47	0.48	0.48	0.48
27	0.40	0.41	0.42	0.44	0.46	0.48	0.50	0.52	0.54	0.54	0.55	0.56	0.56	0.56
28	0.46	0.47	0.49	0.51	0.54	0.56	0.58	0.60	0.61	0.62	0.63	0.64	0.64	0.64
29	0.54	0.55	0.56	0.59	0.61	0.63	0.66	0.68	0.70	0.70	0.71	0.72	0.72	0.72
30	0.61	0.62	0.63	0.66	0.68	0.71	0.73	0.76	0.78	0.78	0.79	0.80	0.80	0.81
35	0.99	1.01	1.02	1.06	1.10	1.13	1.16	1.18	1.20	1.21	1.22	1.22	1.23	1.22
40	1.42	1.45	1.47	1.51	1.54	1.57	1.60	1.62	1.64	1.65	1.65	1.65	1.66	1.65
45	1.91	1.94	1.96	2.00	2.03	2.05	2.07	2.09	2.10	2.10	2.10	2.10	2.10	2.08
50	2.46	2.48	2.50	2.53	2.56	2.57	2.58	2.59	2.59	2.58	2.58	2.57	2.56	2.52
55	3.05	3.07	3.09	3.12	3.12	3.12	3.12	3.11	3.10	3.08	3.07	3.05	3.03	2.97
60	3.69	3.72	3.73	3.73	3.72	3.70	3.67	3.65	3.62	3.60	3.57	3.54	3.50	3.43
27.5	0.43	0.44	0.46	0.48	0.50	0.52	0.54	0.56	0.58	0.58	0.59	0.60	0.60	0.60

<sup>1</sup> Wiss., Abh. Kaiserliche Normal-Eichungs-Kommission, Vol. 2, 1900, p. 140.

5 *Domke's table of apparent specific gravity of sucrose solutions at 20°C.<sup>1</sup>*

Calculated from the tables of the Kaiserliche Normal-Eichungs-Kommission and accepted by the International Commission for Unifying Methods of Sugar Analysis.

DEGREES RIX OR PER CENT BY WEIGHT OF SUCROSE	.0	.1	.2	.3	.4	.5	.6	.7	.8	.9
0	1.0000	1.0004	1.0008	1.0012	1.0016	1.0019	1.0023	1.0027	1.0031	1.0035
1	1.0039	1.0043	1.0047	1.0051	1.0055	1.0058	1.0062	1.0066	1.0070	1.0074
2	1.0078	1.0082	1.0086	1.0090	1.0094	1.0098	1.0102	1.0106	1.0109	1.0113
3	1.0117	1.0121	1.0125	1.0129	1.0133	1.0137	1.0141	1.0145	1.0149	1.0153
4	1.0157	1.0161	1.0165	1.0169	1.0173	1.0177	1.0181	1.0185	1.0189	1.0193
5	1.0197	1.0201	1.0205	1.0209	1.0213	1.0217	1.0221	1.0225	1.0229	1.0233
6	1.0237	1.0241	1.0245	1.0249	1.0253	1.0257	1.0261	1.0265	1.0269	1.0273
7	1.0277	1.0281	1.0285	1.0289	1.0294	1.0298	1.0302	1.0306	1.0310	1.0314
8	1.0318	1.0322	1.0326	1.0330	1.0334	1.0338	1.0343	1.0347	1.0351	1.0355
9	1.0359	1.0363	1.0367	1.0371	1.0375	1.0380	1.0384	1.0388	1.0392	1.0396
10	1.0400	1.0404	1.0409	1.0413	1.0417	1.0421	1.0425	1.0429	1.0433	1.0438
11	1.0442	1.0446	1.0450	1.0454	1.0459	1.0463	1.0467	1.0471	1.0475	1.0480
12	1.0484	1.0488	1.0492	1.0496	1.0501	1.0505	1.0509	1.0513	1.0517	1.0522
13	1.0526	1.0530	1.0534	1.0539	1.0543	1.0547	1.0551	1.0556	1.0560	1.0564
14	1.0568	1.0573	1.0577	1.0581	1.0585	1.0589	1.0594	1.0598	1.0603	1.0607
15	1.0611	1.0615	1.0620	1.0624	1.0628	1.0633	1.0637	1.0641	1.0646	1.0650
16	1.0654	1.0659	1.0663	1.0667	1.0672	1.0676	1.0680	1.0685	1.0689	1.0693
17	1.0698	1.0702	1.0706	1.0711	1.0715	1.0719	1.0724	1.0728	1.0733	1.0737
18	1.0741	1.0746	1.0750	1.0755	1.0759	1.0763	1.0768	1.0772	1.0777	1.0781
19	1.0785	1.0790	1.0794	1.0799	1.0803	1.0807	1.0812	1.0816	1.0821	1.0825
20	1.0830	1.0834	1.0839	1.0843	1.0848	1.0852	1.0856	1.0861	1.0865	1.0870
21	1.0874	1.0879	1.0883	1.0888	1.0892	1.0897	1.0901	1.0905	1.0910	1.0915
22	1.0919	1.0924	1.0928	1.0933	1.0937	1.0942	1.0946	1.0951	1.0956	1.0960
23	1.0965	1.0969	1.0974	1.0978	1.0983	1.0987	1.0992	1.0997	1.1001	1.1006
24	1.1010	1.1015	1.1020	1.1024	1.1029	1.1033	1.1038	1.1043	1.1047	1.1052
25	1.1056	1.1061	1.1066	1.1070	1.1075	1.1079	1.1084	1.1089	1.1093	1.1098
26	1.1103	1.1107	1.1112	1.1117	1.1121	1.1126	1.1131	1.1135	1.1140	1.1145
27	1.1149	1.1154	1.1159	1.1163	1.1168	1.1173	1.1178	1.1182	1.1187	1.1192
28	1.1196	1.1201	1.1206	1.1210	1.1215	1.1220	1.1225	1.1229	1.1234	1.1239
29	1.1244	1.1248	1.1253	1.1258	1.1263	1.1267	1.1272	1.1277	1.1282	1.1287
30	1.1291	1.1296	1.1301	1.1306	1.1311	1.1315	1.1320	1.1325	1.1330	1.1334
31	1.1339	1.1344	1.1349	1.1354	1.1359	1.1363	1.1368	1.1373	1.1378	1.1383
32	1.1388	1.1393	1.1397	1.1402	1.1407	1.1412	1.1417	1.1422	1.1427	1.1432
33	1.1436	1.1441	1.1446	1.1451	1.1456	1.1461	1.1466	1.1471	1.1476	1.1481
34	1.1486	1.1490	1.1495	1.1500	1.1505	1.1510	1.1515	1.1520	1.1525	1.1530
35	1.1535	1.1540	1.1545	1.1550	1.1555	1.1560	1.1565	1.1570	1.1575	1.1580
36	1.1585	1.1590	1.1595	1.1600	1.1605	1.1610	1.1615	1.1620	1.1625	1.1630
37	1.1635	1.1640	1.1645	1.1650	1.1655	1.1660	1.1665	1.1670	1.1675	1.1680
38	1.1685	1.1690	1.1696	1.1701	1.1706	1.1711	1.1716	1.1721	1.1726	1.1731
39	1.1736	1.1741	1.1746	1.1752	1.1757	1.1762	1.1767	1.1772	1.1777	1.1782
40	1.1787	1.1793	1.1798	1.1803	1.1808	1.1813	1.1818	1.1824	1.1829	1.1834
41	1.1839	1.1844	1.1849	1.1855	1.1860	1.1865	1.1870	1.1875	1.1881	1.1886
42	1.1891	1.1896	1.1901	1.1907	1.1912	1.1917	1.1922	1.1928	1.1933	1.1938
43	1.1943	1.1949	1.1954	1.1959	1.1964	1.1970	1.1975	1.1980	1.1985	1.1991
44	1.1996	1.2001	1.2007	1.2012	1.2017	1.2023	1.2028	1.2033	1.2039	1.2044

<sup>1</sup> Z. Ver. deut. Zucker-Ind., 62, 306 (1912).

*Domke's table of apparent specific gravity of sucrose solutions at 20°C.—Concluded. 5*

DEGREES BAUX OR PER CENT BY WEIGHT OF SUCROSE	.0	.1	.2	.3	.4	.5	.6	.7	.8	.9
45	1.2049	1.2054	1.2060	1.2065	1.2070	1.2076	1.2081	1.2087	1.2092	1.2097
46	1.2102	1.2108	1.2113	1.2118	1.2124	1.2129	1.2135	1.2140	1.2146	1.2151
47	1.2156	1.2162	1.2167	1.2173	1.2178	1.2184	1.2189	1.2194	1.2200	1.2205
48	1.2211	1.2216	1.2222	1.2227	1.2232	1.2238	1.2243	1.2249	1.2254	1.2260
49	1.2265	1.2271	1.2276	1.2282	1.2287	1.2293	1.2298	1.2304	1.2309	1.2315
50	1.2320	1.2326	1.2331	1.2337	1.2342	1.2348	1.2353	1.2359	1.2364	1.2370
51	1.2376	1.2381	1.2387	1.2392	1.2398	1.2403	1.2409	1.2415	1.2420	1.2426
52	1.2431	1.2437	1.2442	1.2448	1.2454	1.2459	1.2465	1.2471	1.2476	1.2482
53	1.2487	1.2493	1.2499	1.2504	1.2510	1.2516	1.2521	1.2527	1.2533	1.2538
54	1.2544	1.2550	1.2555	1.2561	1.2567	1.2572	1.2578	1.2584	1.2589	1.2595
55	1.2601	1.2606	1.2612	1.2618	1.2624	1.2629	1.2635	1.2641	1.2647	1.2652
56	1.2658	1.2664	1.2670	1.2675	1.2681	1.2687	1.2693	1.2698	1.2704	1.2710
57	1.2716	1.2721	1.2727	1.2733	1.2739	1.2745	1.2750	1.2756	1.2762	1.2768
58	1.2774	1.2779	1.2785	1.2791	1.2797	1.2803	1.2809	1.2815	1.2821	1.2826
59	1.2832	1.2838	1.2844	1.2850	1.2856	1.2861	1.2867	1.2873	1.2879	1.2885
60	1.2891	1.2897	1.2903	1.2909	1.2914	1.2920	1.2926	1.2932	1.2938	1.2944
61	1.2950	1.2956	1.2962	1.2968	1.2974	1.2980	1.2986	1.2992	1.2998	1.3004
62	1.3010	1.3015	1.3021	1.3027	1.3033	1.3039	1.3045	1.3051	1.3057	1.3063
63	1.3069	1.3075	1.3081	1.3087	1.3093	1.3100	1.3106	1.3112	1.3118	1.3124
64	1.3130	1.3136	1.3142	1.3148	1.3154	1.3160	1.3166	1.3172	1.3178	1.3184
65	1.3190	1.3197	1.3203	1.3209	1.3215	1.3221	1.3227	1.3233	1.3239	1.3245
66	1.3252	1.3258	1.3264	1.3270	1.3276	1.3282	1.3288	1.3295	1.3301	1.3307
67	1.3313	1.3319	1.3325	1.3332	1.3338	1.3344	1.3350	1.3356	1.3363	1.3369
68	1.3375	1.3381	1.3387	1.3394	1.3400	1.3406	1.3412	1.3418	1.3425	1.3431
69	1.3437	1.3443	1.3450	1.3456	1.3462	1.3468	1.3475	1.3481	1.3487	1.3494
70	1.3500	1.3506	1.3512	1.3519	1.3525	1.3531	1.3538	1.3544	1.3550	1.3557
71	1.3563	1.3569	1.3575	1.3582	1.3588	1.3594	1.3601	1.3607	1.3614	1.3620
72	1.3626	1.3633	1.3639	1.3645	1.3652	1.3658	1.3664	1.3671	1.3677	1.3684
73	1.3690	1.3696	1.3703	1.3709	1.3716	1.3722	1.3729	1.3735	1.3741	1.3748
74	1.3754	1.3761	1.3767	1.3774	1.3780	1.3786	1.3793	1.3799	1.3806	1.3812
75	1.3819	1.3825	1.3832	1.3838	1.3845	1.3851	1.3858	1.3864	1.3871	1.3877
76	1.3884	1.3890	1.3897	1.3903	1.3910	1.3916	1.3923	1.3929	1.3936	1.3942
77	1.3949	1.3955	1.3962	1.3969	1.3975	1.3982	1.3988	1.3995	1.4001	1.4008
78	1.4015	1.4021	1.4028	1.4034	1.4041	1.4048	1.4054	1.4061	1.4067	1.4074
79	1.4081	1.4087	1.4094	1.4101	1.4107	1.4114	1.4121	1.4127	1.4134	1.4140
80	1.4147	1.4154	1.4160	1.4167	1.4174	1.4180	1.4187	1.4194	1.4201	1.4207
81	1.4214	1.4221	1.4227	1.4234	1.4241	1.4247	1.4254	1.4261	1.4268	1.4274
82	1.4281	1.4288	1.4295	1.4301	1.4308	1.4315	1.4322	1.4328	1.4335	1.4342
83	1.4349	1.4355	1.4362	1.4369	1.4376	1.4383	1.4389	1.4396	1.4403	1.4410
84	1.4417	1.4423	1.4430	1.4437	1.4444	1.4451	1.4458	1.4464	1.4471	1.4478
85	1.4485	1.4492	1.4499	1.4505	1.4512	1.4519	1.4526	1.4533	1.4540	1.4547
86	1.4554	1.4560	1.4567	1.4574	1.4581	1.4588	1.4595	1.4602	1.4609	1.4616
87	1.4623	1.4629	1.4636	1.4643	1.4650	1.4657	1.4664	1.4671	1.4678	1.4685
88	1.4692	1.4699	1.4706	1.4713	1.4720	1.4727	1.4734	1.4741	1.4748	1.4755
89	1.4762	1.4769	1.4776	1.4783	1.4790	1.4797	1.4804	1.4811	1.4818	1.4825
90	1.4832									



6 Schönrock's table for determining the percentage of sucrose in sugar solutions by means of the Abbé refractometer.<sup>1</sup>

REFRACTIVE INDEX AT 20°	SUCROSE, PER CENT	REFRACTIVE INDEX AT 20°	SUCROSE, PER CENT	REFRACTIVE INDEX AT 20°	SUCROSE, PER CENT	REFRACTIVE INDEX AT 20°	SUCROSE, PER CENT	REFRACTIVE INDEX AT 20°	SUCROSE, PER CENT
1.3330	0.0	1.3464	9.0	1.3606	18.0	1.3758	27.0	1.3920	36.0
.3333	0.2	.3467	9.2	.3609	18.2	.3761	27.2	.3924	36.2
.3336	0.4	.3470	9.4	.3612	18.4	.3765	27.4	.3928	36.4
.3338	0.6	.3473	9.6	.3616	18.6	.3768	27.6	.3931	36.6
.3341	0.8	.3476	9.8	.3619	18.8	.3772	27.8	.3935	36.8
.3344	1.0	.3479	10.0	.3622	19.0	.3775	28.0	.3939	37.0
.3347	1.2	.3482	10.2	.3625	19.2	.3779	28.2	.3943	37.2
.3350	1.4	.3485	10.4	.3629	19.4	.3782	28.4	.3947	37.4
.3353	1.6	.3488	10.6	.3632	19.6	.3886	28.6	.3950	37.6
.3356	1.8	.3491	10.8	.3636	19.8	.3789	28.8	.3954	37.8
.3359	2.0	.3494	11.0	.3639	20.0	.3793	29.0	.3958	38.0
.3362	2.2	.3497	11.2	.3642	20.2	.3797	29.2	.3962	38.2
.3365	2.4	.3500	11.4	.3645	20.4	.3800	29.4	.3966	38.4
.3368	2.6	.3504	11.6	.3649	20.6	.3804	29.6	.3970	38.6
.3371	2.8	.3507	11.8	.3652	20.8	.3807	29.8	.3974	38.8
.3374	3.0	.3510	12.0	.3655	21.0	.3811	30.0	.3978	39.0
.3377	3.2	.3513	12.2	.3658	21.2	.3815	30.2	.3982	39.2
.3380	3.4	.3516	12.4	.3662	21.4	.3818	30.4	.3986	39.4
.3382	3.6	.3520	12.6	.3665	21.6	.3822	30.6	.3989	39.6
.3385	3.8	.3523	12.8	.3669	21.8	.3825	30.8	.3993	39.8
.3388	4.0	.3526	13.0	.3672	22.0	.3829	31.0	.3997	40.0
.3391	4.2	.3529	13.2	.3675	22.2	.3833	31.2	.4001	40.2
.3394	4.4	.3532	13.4	.3679	22.4	.3836	31.4	.4005	40.4
.3397	4.6	.3535	13.6	.3682	22.6	.3840	31.6	.4008	40.6
.3400	4.8	.3538	13.8	.3686	22.8	.3843	31.8	.4012	40.8
.3403	5.0	.3541	14.0	.3689	23.0	.3847	32.0	.4016	41.0
.3406	5.2	.3544	14.2	.3692	23.2	.3851	32.2	.4020	41.2
.3409	5.4	.3547	14.4	.3696	23.4	.3854	32.4	.4024	41.4
.3412	5.6	.3551	14.6	.3699	23.6	.3858	32.6	.4028	41.6
.3415	5.8	.3554	14.8	.3703	23.8	.3861	32.8	.4032	41.8
.3418	6.0	.3557	15.0	.3706	24.0	.3865	33.0	.4036	42.0
.3421	6.2	.3560	15.2	.3709	24.2	.3869	33.2	.4040	42.2
.3424	6.4	.3563	15.4	.3713	24.4	.3872	33.4	.4044	42.4
.3427	6.6	.3567	15.6	.3716	24.6	.3876	33.6	.4048	42.6
.3430	6.8	.3570	15.8	.3720	24.8	.3879	33.8	.4052	42.8
.3433	7.0	.3573	16.0	.3723	25.0	.3883	34.0	.4056	43.0
.3436	7.2	.3576	16.2	.3726	25.2	.3887	34.2	.4060	43.2
.3439	7.4	.3580	16.4	.3730	25.4	.3891	34.4	.4064	43.4
.3442	7.6	.3583	16.6	.3733	25.6	.3894	34.6	.4068	43.6
.3445	7.8	.3587	16.8	.3737	25.8	.3898	34.8	.4072	43.8
.3448	8.0	.3590	17.0	.3740	26.0	.3902	35.0	.4076	44.0
.3451	8.2	.3593	17.2	.3744	26.2	.3906	35.2	.4080	44.2
.3454	8.4	.3596	17.4	.3747	26.4	.3909	35.4	.4084	44.4
.3458	8.6	.3600	17.6	.3751	26.6	.3913	35.6	.4088	44.6
.3461	8.8	.3603	17.8	.3754	26.8	.3916	35.8	.4092	44.8

<sup>1</sup> Z. Ver. deut. Zucker-Ind., 48, 421 (1911).

## REFERENCE TABLES

XLIII

*Schönrock's table.*—Concluded.

6

REFRACTIVE INDEX AT 20°	SUCROSE, PER CENT	REFRACTIVE INDEX AT 20°	SUCROSE, PER CENT	REFRACTIVE INDEX AT 20°	SUCROSE, PER CENT	REFRACTIVE INDEX AT 20°	SUCROSE, PER CENT	REFRACTIVE INDEX AT 20°	SUCROSE, PER CENT
1.4096	45.0	1.4264	53.0	1.4441	61.0	1.4627	69.0	1.4825	77.0
.4100	45.2	.4268	53.2	.4446	61.2	.4631	69.2	.4830	77.2
.4104	45.4	.4272	53.4	.4450	61.4	.4636	69.4	.4835	77.4
.4109	45.6	.4277	53.6	.4455	61.6	.4641	69.6	.4840	77.6
.4113	45.8	.4281	53.8	.4459	61.8	.4646	69.8	.4845	77.8
.4117	46.0	.4285	54.0	.4464	62.0	.4651	70.0 <sup>1</sup>	.4850	78.0
.4121	46.2	.4289	54.2	.4468	62.2	.4656	70.2	.4855	78.2
.4125	46.4	.4294	54.4	.4473	62.4	.4661	70.4	.4860	78.4
.4129	46.6	.4298	54.6	.4477	62.6	.4666	70.6	.4865	78.6
.4133	46.8	.4303	54.8	.4482	62.8	.4671	70.8	.4871	78.8
.4137	47.0	.4307	55.0	.4486	63.0	.4676	71.0	.4876	79.0
.4141	47.2	.4311	55.2	.4491	63.2	.4681	71.2	.4881	79.2
.4145	47.4	.4316	55.4	.4495	63.4	.4685	71.4	.4886	79.4
.4150	47.6	.4320	55.6	.4500	63.6	.4690	71.6	.4891	79.6
.4154	47.8	.4325	55.8	.4504	63.8	.4695	71.8	.4896	79.8
.4158	48.0	.4329	56.0	.4509	64.0	.4700	72.0	.4901	80.0
.4162	48.2	.4333	56.2	.4514	64.2	.4705	72.2	.4906	80.2
.4166	48.4	.4338	56.4	.4518	64.4	.4710	72.4	.4912	80.4
.4171	48.6	.4342	56.6	.4523	64.6	.4715	72.6	.4917	80.6
.4175	48.8	.4347	56.8	.4527	64.8	.4720	72.8	.4922	80.8
.4179	49.0	.4351	57.0	.4532	65.0	.4725	73.0	.4927	81.0
.4183	49.2	.4355	57.2	.4537	65.2	.4730	73.2	.4933	81.2
.4187	49.4	.4360	57.4	.4541	65.4	.4735	73.4	.4938	81.4
.4192	49.6	.4364	57.6	.4546	65.6	.4740	73.6	.4943	81.6
.4196	49.8	.4369	57.8	.4550	65.8	.4744	73.8	.4949	81.8
.4200	50.0	.4373	58.0	.4555	66.0	.4749	74.0	.4954	82.0
.4204	50.2	.4378	58.2	.4560	66.2	.4754	74.2	.4959	82.2
.4208	50.4	.4382	58.4	.4565	66.4	.4759	74.4	.4964	82.4
.4213	50.6	.4387	58.6	.4569	66.6	.4764	74.6	.4970	82.6
.4217	50.8	.4391	58.8	.4574	66.8	.4769	74.8	.4975	82.8
.4221	51.0	.4396	59.0	.4579	67.0	.4774	75.0	.4980	83.0
.4225	51.2	.4400	59.2	.4584	67.2	.4779	75.2	.4985	83.2
.4229	51.4	.4405	59.4	.4589	67.4	.4784	75.4	.4991	83.4
.4234	51.6	.4409	59.6	.4593	67.6	.4789	75.6	.4996	83.6
.4238	51.8	.4414	59.8	.4598	67.8	.4794	75.8	.5001	83.8
.4242	52.0	.4418	60.0	.4603	68.0	.4799	76.0	.5007	84.0
.4246	52.2	.4423	60.2	.4607	68.2	.4804	76.2	.5012	84.2
.4251	52.4	.4427	60.4	.4612	68.4	.4810	76.4	.5017	84.4
.4255	52.6	.4432	60.6	.4617	68.6	.4815	76.6	.5022	84.6
.4260	52.8	.4436	60.8	.4622	68.8	.4820	76.8	.5028	84.8
								.5033	85.0

<sup>1</sup> The values from 70 to 85% are taken from Main's table, *Int. Sugar J.*, 9, 471 (1907).<sup>2</sup>

7 Table of corrections for determining the percentage of sucrose in sugar solutions by means of either the Abbé or immersion refractometer when the readings are made at temperatures other than 20°C.<sup>1</sup>

SUGAR %	0	5	10	15	20	25	30	40	50	60	70	80
Temp.	To be subtracted from the per cent of sucrose											
15	0.28	0.30	0.31	0.32	0.34	0.35	0.35	0.37	0.38	0.39	0.39	0.40
16	.23	.24	.25	.26	.27	.28	.29	.30	.30	.31	.31	.32
17	.17	.18	.19	.20	.21	.21	.22	.22	.23	.23	.24	.24
18	.12	.13	.13	.13	.14	.14	.15	.15	.15	.16	.16	.16
19	.06	.06	.07	.07	.07	.07	.07	.08	.08	.08	.08	.08
	To be added to the per cent of sucrose											
21	.06	.07	.07	.07	.07	.07	.07	.08	.08	.08	.08	.08
22	.13	.13	.14	.14	.14	.15	.15	.15	.16	.16	.16	.16
23	.20	.20	.21	.21	.22	.22	.23	.23	.24	.24	.24	.24
24	.27	.27	.28	.29	.30	.30	.31	.31	.32	.32	.32	.32
25	.34	.35	.36	.37	.37	.38	.38	.39	.40	.40	.40	.40
26	.41	.42	.44	.44	.45	.46	.46	.47	.48	.48	.48	.48
27	.49	.50	.51	.52	.53	.54	.54	.55	.56	.56	.56	.56
28	.57	.58	.59	.61	.61	.62	.63	.64	.64	.64	.64	.64
29	.65	.66	.68	.69	.70	.71	.71	.72	.72	.72	.72	.72
30	.73	.75	.76	.77	.78	.79	.80	.81	.81	.81	.80	.80

<sup>1</sup> The values in this table were calculated by C. F. Snyder and J. A. Mathews from the temperature coefficients of Schöorrock, *Z. Ver. deut. Zucker-Ind.*, 48, 425 (1911).

Table for determining the percentage of sucrose in sugar solutions from the readings of the Zeiss immersion refractometer at 20°C.<sup>1</sup>

SCALE READING <sup>2</sup> 20°C.	$n_D^{20}$	SUCROSE PER CENT	SCALE READING 20°C.	$n_D^{20}$	SUCROSE PER CENT	SCALE READING 20°C.	$n_D^{20}$	SUCROSE PER CENT
14.47	1.33299	0	45	1.34463	7.91	76	1.35606	15.24
15	3320	0.15	46	4500	8.15	77	5642	15.47
16	3353	0.41	47	4537	8.39	78	5678	15.69
17	3397	0.68	48	4575	8.64	79	5714	15.91
18	3435	0.94	49	4612	8.89	80	5750	16.14
19	3474	1.21	50	4650	9.13	81	5786	16.36
20	3513	1.48	51	4687	9.38	82	5822	16.58
21	3551	1.74	52	4724	9.62	83	5858	16.81
22	3590	2.01	53	4761	9.86	84	5894	17.03
23	3628	2.27	54	4798	10.10	85	5930	17.25
24	3667	2.54	55	4836	10.34	86	5966	17.47
25	3705	2.80	56	4873	10.58	87	6002	17.69
26	3743	3.07	57	4910	10.82	88	6038	17.91
27	3781	3.33	58	4947	11.06	89	6074	18.12
28	3820	3.59	59	4984	11.30	90	6109	18.34
29	3858	3.85	60	5021	11.54	91	6145	18.56
30	3896	4.11	61	5058	11.78	92	6181	18.78
31	3934	4.36	62	5095	12.01	93	6217	19.00
32	3972	4.62	63	5132	12.25	94	6252	19.21
33	4010	4.88	64	5169	12.48	95	6287	19.42
34	4048	5.14	65	5205	12.72	96	6323	19.63
35	4086	5.40	66	5242	12.95	97	6359	19.85
36	4124	5.65	67	5279	13.18	98	6394	20.06
37	4162	5.91	68	5316	13.41	99	6429	20.27
38	4199	6.16	69	5352	13.64	100	6464	20.48
39	4237	6.41	70	5388	13.87	101	6500	20.69
40	4275	6.66	71	5425	14.10	102	6535	20.90
41	4313	6.91	72	5461	14.33	103	6570	21.11
42	4350	7.16	73	5497	14.56	104	6605	21.32
43	4388	7.41	74	5533	14.79	105	6640	21.53
44	4426	7.66	75	5569	15.01			

<sup>1</sup> The values in this table were calculated by J. A. Mathews from the five-place indices of Schönrock as given by Landt, *Z. Ver. deut. Zucker-Ind.*, 83, 692 (1933).

<sup>2</sup> The scale readings refer only to the scale of arbitrary units proposed by Pulfrich, *Z. anorg. Chem.*, p. 1168 (1899). According to this scale 14.5=1.33300, 50.0=1.34650, and 100.0=1.36464. If the immersion refractometer used is calibrated according to another arbitrary scale, the readings must be converted into refractive indices before this table is used to determine the percentage of sugar.

9 Munson and Walker's table for calculating dextrose, invert sugar alone, invert sugar in the presence of sucrose (0.4 gram and 2 grams total sugar), lactose, lactose and sucrose (2 mixtures), and maltose (crystallized).<sup>1</sup>  
(Expressed in milligrams.)

CUPROUS OXIDE (Cu <sub>2</sub> O)	COPPER (Cu)	DEXTRINE (d-ALCOSE)	INVERT SUGAR	INVERT SUGAR AND SUCROSE		LACTOSE C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> + H <sub>2</sub> O	LACTOSE AND SUCROSE		MALTOSE C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> + H <sub>2</sub> O	CUPROUS OXIDE (Cu <sub>2</sub> O)
				0.4 gram total sugar	2 grams total sugar		1 lactose, 4 sucrose	1 lactose, 12 sucrose		
10	8.9	4.0	4.5	1.6	.....	6.3	6.1	.....	6.2	10
12	10.7	4.9	5.4	2.5	.....	7.5	7.3	.....	7.9	12
14	12.4	5.7	6.3	3.4	.....	8.8	8.5	.....	9.5	14
16	14.2	6.6	7.2	4.3	.....	10.0	9.7	.....	11.2	16
18	16.0	7.5	8.1	5.2	.....	11.3	10.9	.....	12.9	18
20	17.8	8.3	8.9	6.1	.....	12.5	12.1	.....	14.6	20
22	19.5	9.2	9.8	7.0	.....	13.8	13.3	.....	16.2	22
24	21.3	10.0	10.7	7.9	.....	15.0	14.5	.....	17.9	24
26	23.1	10.9	11.6	8.8	.....	16.3	15.8	.....	19.6	26
28	24.9	11.8	12.5	9.7	.....	17.6	17.0	.....	21.2	28
30	26.6	12.6	13.4	10.7	4.3	18.8	18.2	.....	22.9	30
32	28.4	13.5	14.3	11.6	5.2	20.1	19.4	.....	24.6	32
34	30.2	14.3	15.2	12.5	6.1	21.4	20.7	.....	26.2	34
36	32.0	15.2	16.1	13.4	7.0	22.8	22.0	.....	27.9	36
38	33.8	16.1	16.9	14.3	7.9	24.2	23.3	.....	29.6	38
40	35.5	16.9	17.8	15.2	8.8	25.5	24.7	.....	31.3	40
42	37.3	17.8	18.7	16.1	9.7	26.6	26.0	.....	32.9	42
44	39.1	18.7	19.6	17.0	10.7	28.3	27.3	.....	34.6	44
46	40.9	19.6	20.5	17.9	11.6	29.6	28.6	.....	36.3	46
48	42.6	20.4	21.4	18.8	12.5	31.0	30.0	.....	37.9	48
50	44.4	21.3	22.3	19.7	13.4	32.3	31.3	.....	39.6	50
52	46.2	22.2	23.2	20.7	14.3	33.7	32.6	.....	41.3	52
54	48.0	23.0	24.1	21.6	15.2	35.1	34.0	.....	42.9	54
56	49.7	23.9	25.0	22.5	16.2	36.4	35.3	.....	44.6	56
58	51.5	24.8	25.9	23.4	17.1	37.8	36.6	.....	46.3	58
60	53.3	25.6	26.8	24.3	18.0	39.2	37.9	.....	48.0	60
62	55.1	26.5	27.7	25.2	18.9	40.5	39.3	.....	49.6	62
64	56.8	27.4	28.6	26.2	19.8	41.9	40.6	.....	51.3	64
66	58.6	28.3	29.5	27.1	20.8	43.3	41.9	.....	53.0	66
68	60.4	29.2	30.4	28.0	21.7	44.7	43.3	40.7	54.6	68
70	62.2	30.0	31.3	28.9	22.6	46.0	44.6	41.9	56.3	70
72	64.0	30.9	32.3	29.8	23.5	47.4	45.9	43.1	58.0	72
74	65.7	31.8	33.2	30.8	24.5	48.8	47.3	44.2	59.6	74
76	67.5	32.7	34.1	31.7	25.4	50.1	48.6	45.4	61.3	76
78	69.3	33.6	35.0	32.6	26.3	51.5	49.9	46.6	63.0	78
80	71.1	34.4	35.9	33.5	27.3	52.9	51.3	47.8	64.6	80
82	72.8	35.3	36.8	34.5	28.2	54.2	52.6	49.0	66.3	82
84	74.6	36.2	37.7	35.4	29.1	55.6	53.9	50.1	68.0	84
86	76.4	37.1	38.6	36.3	30.0	57.0	55.3	51.3	69.7	86
88	78.2	38.0	39.5	37.2	31.0	58.4	56.6	52.5	71.3	88

<sup>1</sup> U. S. Bur. Standards Circ. 44, p. 139. The columns headed "Lactose" and "Lactose and Sucrose" were taken from "Methods of Sugar Analysis and Allied Determinations" by Arthur Given.

*Munson and Walker's table.*—Continued.  
(Expressed in milligrams.)

9

CUPROUS OXIDE (Cu <sub>2</sub> O)	COPPER (Cu)	DEXTROSE (d-glucose)	INVERT SUGAR	INVERT SUGAR AND SUCROSE		LACTOSE C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> +H <sub>2</sub> O	LACTOSE AND SUCROSE		MALTOSE C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> +H <sub>2</sub> O	CUPROUS OXIDE (Cu <sub>2</sub> O)
				0.4 gram total sugar	2 grams total sugar		1 lactose, 4 glucose	1 lactose, 12 glucose		
90	79.9	38.9	40.4	38.2	31.9	59.7	57.9	53.7	73.0	90
92	81.7	39.8	41.4	39.1	32.8	61.1	59.3	54.9	74.7	92
94	83.5	40.6	42.3	40.0	33.8	62.5	60.6	56.0	76.3	94
96	85.3	41.5	43.2	41.0	34.7	63.8	61.9	57.2	78.0	96
98	87.1	42.4	44.1	41.9	35.6	65.2	63.3	58.4	79.7	98
100	88.8	43.3	45.0	42.8	36.6	66.6	64.6	59.6	81.3	100
102	90.6	44.2	46.0	43.8	37.5	68.0	66.0	60.8	83.0	102
104	92.4	45.1	46.9	44.7	38.5	69.3	67.3	62.0	84.7	104
106	94.2	46.0	47.8	45.6	39.4	70.7	68.6	63.2	86.3	106
108	95.9	46.9	48.7	46.6	40.3	72.1	70.0	64.4	88.0	108
110	97.7	47.8	49.6	47.5	41.3	73.5	71.3	65.6	89.7	110
112	99.5	48.7	50.6	48.4	42.2	74.8	72.6	66.7	91.3	112
114	101.3	49.6	51.5	49.4	43.2	76.2	74.0	67.9	93.0	114
116	103.0	50.5	52.4	50.3	44.1	77.6	75.3	69.1	94.7	116
118	104.8	51.4	53.3	51.2	45.0	79.0	76.7	70.3	96.4	118
120	106.6	52.3	54.3	52.2	46.0	80.3	78.0	71.5	98.0	120
122	108.4	53.2	55.2	53.1	46.9	81.7	79.3	72.7	99.7	122
124	110.1	54.1	56.1	54.1	47.9	83.1	80.7	73.9	101.4	124
126	111.9	55.0	57.0	55.0	48.8	84.5	82.0	75.1	103.0	126
128	113.7	55.9	58.0	55.9	49.8	85.8	83.4	76.3	104.7	128
130	115.5	56.8	58.9	56.9	50.7	87.2	84.7	77.5	106.4	130
132	117.3	57.7	59.8	57.8	51.7	88.6	86.0	78.7	108.0	132
134	119.0	58.6	60.8	58.8	52.6	90.0	87.4	79.7	109.7	134
136	120.8	59.5	61.7	59.7	53.6	91.3	88.7	81.1	111.4	136
138	122.6	60.4	62.6	60.7	54.5	92.7	90.1	82.3	113.0	138
140	124.4	61.3	63.6	61.6	55.5	94.1	91.4	83.5	114.7	140
142	126.1	62.2	64.5	62.6	56.4	95.5	92.8	84.7	116.4	142
144	127.9	63.1	65.4	63.5	57.4	96.8	94.1	85.9	118.0	144
146	129.7	64.0	66.4	64.5	58.3	98.2	95.4	87.1	119.7	146
148	131.5	65.0	67.3	65.4	59.3	99.6	96.8	88.3	121.4	148
150	133.2	65.9	68.3	66.4	60.2	101.0	98.1	89.5	123.0	150
152	135.0	66.8	69.2	67.3	61.2	102.3	99.5	90.8	124.7	152
154	136.8	67.7	70.1	68.3	62.1	103.7	100.8	92.0	126.4	154
156	138.6	68.6	71.1	69.2	63.1	105.1	102.2	93.2	128.0	156
158	140.3	69.5	72.0	70.2	64.1	106.5	103.5	94.4	129.7	158
160	142.1	70.4	73.0	71.2	65.0	107.9	104.8	95.6	131.4	160
162	143.9	71.4	73.9	72.1	66.0	109.2	106.2	96.8	133.0	162
164	145.7	72.3	74.9	73.1	66.9	110.6	107.5	98.0	134.7	164
166	147.5	73.2	75.8	74.0	67.9	112.0	108.9	99.2	136.4	166
168	149.2	74.1	76.8	75.0	68.9	113.4	110.2	100.4	138.0	168

Munson and Walker's table.—Continued.  
(Expressed in milligrams.)

CUPROUS OXIDE (Cu <sub>2</sub> O)	COPPER (Cu)	DEXTROSE (β-DGLUCOSE)	INVERT SUGAR	INVERT SUGAR AND SUCROSE		LACTOSE C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> +H <sub>2</sub> O	LACTOSE AND SUCROSE		MALTOSE C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> +H <sub>2</sub> O	CUPROUS OXIDE (Cu <sub>2</sub> O)
				0.5 gram total sugar	2 grams total sugar		1 lactose, 4 sucrose	1 lactose, 12 sucrose		
170	151.0	75.1	77.7	76.0	69.8	114.8	111.6	101.6	139.7	170
172	152.8	76.0	78.7	76.9	70.8	116.1	112.9	102.8	141.4	172
174	154.6	76.9	79.6	77.9	71.7	117.5	114.3	104.1	143.0	174
176	156.3	77.8	80.6	78.8	72.7	118.9	115.6	105.3	144.7	176
178	158.1	78.8	81.5	79.8	73.7	120.3	117.0	106.5	146.4	178
180	159.9	79.7	82.5	80.8	74.6	121.6	118.3	107.7	148.0	180
182	161.7	80.6	83.4	81.7	75.6	123.1	119.7	108.9	149.7	182
184	163.4	81.5	84.4	82.7	76.6	124.3	121.0	110.1	151.4	184
186	165.2	82.5	85.3	83.7	77.6	125.8	122.4	111.3	153.0	186
188	167.0	83.4	86.3	84.6	78.5	127.2	123.7	112.5	154.7	188
190	168.8	84.3	87.2	85.6	79.5	128.5	125.1	113.8	156.4	190
192	170.5	85.3	88.2	86.6	80.5	129.9	126.4	115.0	158.0	192
194	172.3	86.2	89.2	87.6	81.4	131.3	127.8	116.2	159.7	194
196	174.1	87.1	90.1	88.5	82.4	132.7	129.2	117.4	161.4	196
198	175.9	88.1	91.1	89.5	83.4	134.1	130.5	118.6	163.0	198
200	177.7	89.0	92.0	90.5	84.4	135.4	131.9	119.8	164.7	200
202	179.4	89.9	93.0	91.4	85.3	136.8	133.2	121.0	166.4	202
204	181.2	90.9	94.0	92.4	86.3	138.2	134.6	122.3	168.0	204
206	183.0	91.8	94.9	93.4	87.3	139.6	135.9	123.5	169.7	206
208	184.8	92.8	95.9	94.4	88.3	141.0	137.3	124.7	171.4	208
210	186.5	93.7	96.9	95.4	89.2	142.3	138.6	126.0	173.0	210
212	188.3	94.6	97.8	96.3	90.2	143.7	140.0	127.2	174.7	212
214	190.1	95.6	98.8	97.3	91.2	145.1	141.4	128.4	176.4	214
216	191.9	96.5	99.8	98.3	92.2	146.5	142.7	129.6	178.0	216
218	193.6	97.5	100.8	99.3	93.2	147.9	144.1	130.9	179.7	218
220	195.4	98.4	101.7	100.3	94.2	149.3	145.4	132.1	181.4	220
222	197.2	99.4	102.7	101.2	95.1	150.7	146.8	133.3	183.0	222
224	199.0	100.3	103.7	102.2	96.1	152.0	148.1	134.5	184.7	224
226	200.7	101.3	104.6	103.2	97.1	153.4	149.5	135.8	186.4	226
228	202.5	102.2	105.6	104.2	98.1	154.8	150.8	137.0	188.0	228
230	204.3	103.2	106.6	105.2	99.1	156.2	152.2	138.2	189.7	230
232	206.1	104.1	107.6	106.2	100.1	157.6	153.6	139.4	191.3	232
234	207.9	105.1	108.6	107.2	101.1	159.0	154.9	140.7	193.0	234
236	209.6	106.0	109.5	108.2	102.1	160.3	156.3	141.9	194.7	236
238	211.4	107.0	110.5	109.2	103.1	161.7	157.6	143.2	196.3	238
240	213.2	108.0	111.5	110.1	104.0	163.1	159.0	144.4	198.0	240
242	215.0	108.9	112.5	111.1	105.0	164.5	160.3	145.6	199.7	242
244	216.7	109.9	113.5	112.1	106.0	165.9	161.7	146.9	201.3	244
246	218.5	110.8	114.5	113.1	107.0	167.3	163.1	148.1	203.0	246
248	220.3	111.8	115.4	114.1	108.0	168.7	164.4	149.3	204.7	248

Munson and Walker's table.—Continued.  
(Expressed in milligrams.)

9

CUPROUS OXIDE (Cu <sub>2</sub> O)	COPPER (Cu)	DEXTROSE (d-glucose)	INVERT SUGAR	INVERT SUGAR AND SUCROSE		LACTOSE C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> +H <sub>2</sub> O	LACTOSE AND SUCROSE		MALTOSE C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> +H <sub>2</sub> O	CUPROUS OXIDE (Cu <sub>2</sub> O)
				0.4 gram total sugar	2 grams total sugar		1 lactose, 4 sucrose	1 lactose, 12 sucrose		
250	222.1	112.8	116.4	115.1	109.0	170.1	165.8	150.6	206.3	250
252	223.8	113.7	117.4	116.1	110.0	171.5	167.2	151.8	208.0	252
254	225.6	114.7	118.4	117.1	111.0	172.8	168.5	153.1	209.7	254
256	227.4	115.7	119.4	118.1	112.0	174.2	169.9	154.3	211.3	256
258	229.2	116.6	120.4	119.1	113.0	175.6	171.3	155.5	213.0	258
260	231.0	117.6	121.4	120.1	114.0	177.0	172.6	156.8	214.7	260
262	232.7	118.6	122.4	121.1	115.0	178.4	174.0	158.0	216.3	262
264	234.5	119.5	123.4	122.1	116.0	179.8	175.3	159.3	218.0	264
266	236.3	120.5	124.4	123.1	117.0	181.2	176.7	160.5	219.7	266
268	238.1	121.5	125.4	124.1	118.0	182.6	178.1	161.8	221.3	268
270	239.8	122.5	126.4	125.1	119.0	184.0	179.4	163.0	223.0	270
272	241.6	123.4	127.4	126.2	120.0	185.3	180.8	164.3	224.6	272
274	243.4	124.4	128.4	127.2	121.1	186.7	182.2	165.5	226.3	274
276	245.2	125.4	129.4	128.2	122.1	188.1	183.5	166.8	228.0	276
278	246.9	126.4	130.4	129.2	123.1	189.5	184.9	168.0	229.6	278
280	248.7	127.3	131.4	130.2	124.1	190.9	186.3	169.3	231.3	280
282	250.5	128.3	132.4	131.2	125.1	192.3	187.6	170.5	233.0	282
284	252.3	129.3	133.4	132.2	126.1	193.7	189.0	171.8	234.6	284
286	254.0	130.3	134.4	133.2	127.1	195.1	190.4	173.0	236.3	286
288	255.8	131.3	135.4	134.3	128.1	196.5	191.7	174.3	238.0	288
290	257.6	132.3	136.4	135.3	129.2	197.8	193.1	175.5	239.6	290
292	259.4	133.2	137.4	136.3	130.2	199.2	194.4	176.8	241.3	292
294	261.2	134.2	138.4	137.3	131.2	200.6	195.8	178.1	242.9	294
296	262.9	135.2	139.4	138.3	132.2	202.0	197.2	179.3	244.6	296
298	264.7	136.2	140.5	139.4	133.2	203.4	198.6	180.6	246.3	298
300	266.5	137.2	141.5	140.4	134.2	204.8	199.9	181.8	247.9	300
302	268.3	138.2	142.5	141.4	135.3	206.2	201.3	183.1	249.6	302
304	270.0	139.2	143.5	142.4	136.3	207.6	202.7	184.4	251.3	304
306	271.8	140.2	144.5	143.4	137.3	209.0	204.0	185.6	252.9	306
308	273.6	141.2	145.5	144.5	138.3	210.4	205.4	186.9	254.6	308
310	275.4	142.2	146.6	145.5	139.4	211.8	206.8	188.1	256.3	310
312	277.1	143.2	147.6	146.5	140.4	213.2	208.1	189.4	257.9	312
314	278.9	144.2	148.6	147.6	141.4	214.6	209.5	190.7	259.6	314
316	280.7	145.2	149.6	148.6	142.4	216.0	210.9	191.9	261.2	316
318	282.5	146.2	150.7	149.6	143.5	217.3	212.2	193.2	262.9	318
320	284.2	147.2	151.7	150.7	144.5	218.7	213.6	194.4	264.6	320
322	286.0	148.2	152.7	151.7	145.5	220.1	215.0	195.7	266.2	322
324	287.8	149.2	153.7	152.7	146.6	221.5	216.4	197.0	267.9	324
326	289.6	150.2	154.8	153.8	147.6	222.9	217.7	198.2	269.6	326
328	291.4	151.2	155.8	154.8	148.6	224.3	219.1	199.5	271.2	328



Munson and Walker's table.—Continued.  
(Expressed in milligrams.)

	CURIOUS OXIDE (Cu <sub>2</sub> O)	COPPER (Cu)	DEXTRINE (d-glucose)	INVERT SUGAR	INVERT SUGAR AND SUCROSE		LACTOSE C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> +H <sub>2</sub> O	LACTOSE AND SUCROSE		MALTOSE C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> +H <sub>2</sub> O	CURIOUS OXIDE (Cu <sub>2</sub> O)
					0.4 gram total sugar	2 grams total sugar		1 lactose, 4 su- crose	1 lactose, 12 su- crose		
330	293.1	152.2	156.8	155.8	149.7	225.7	220.5	200.8	272.9	330	
332	294.9	153.2	157.9	156.9	150.7	227.1	221.8	202.0	274.6	332	
334	296.7	154.2	158.9	157.9	151.7	228.5	223.2	203.3	276.2	334	
336	298.5	155.2	159.9	159.0	152.8	229.9	224.6	204.6	277.9	336	
338	300.2	156.3	161.0	160.0	153.8	231.3	226.0	205.9	279.5	338	
340	302.0	157.3	162.0	161.0	154.8	232.7	227.4	207.1	281.2	340	
342	303.8	158.3	163.1	162.1	155.9	234.1	228.7	208.4	282.9	342	
344	305.6	159.3	164.1	163.1	156.9	235.5	230.1	209.7	284.5	344	
346	307.3	160.3	165.1	164.2	158.0	236.9	231.5	211.0	286.2	346	
348	309.1	161.4	166.2	165.2	159.0	238.3	232.9	212.2	287.9	348	
350	310.9	162.4	167.2	166.3	160.1	239.7	234.3	213.5	289.5	350	
352	312.7	163.4	168.3	167.3	161.1	241.1	235.6	214.8	291.2	352	
354	314.4	164.4	169.3	168.4	162.2	242.5	237.0	216.1	292.8	354	
356	316.2	165.4	170.4	169.4	163.2	243.9	238.4	217.3	294.5	356	
358	318.0	166.5	171.4	170.5	164.3	245.3	239.8	218.6	296.2	358	
360	319.8	167.5	172.5	171.5	165.3	246.7	241.2	219.9	297.8	360	
362	321.6	168.5	173.5	172.6	166.4	248.1	242.5	221.2	299.5	362	
364	323.3	169.6	174.6	173.7	167.4	249.5	243.9	222.5	301.2	364	
366	325.1	170.6	175.6	174.7	168.5	250.9	245.3	223.7	302.8	366	
368	326.9	171.6	176.7	175.8	169.5	252.3	246.7	225.0	304.5	368	
370	328.7	172.7	177.7	176.8	170.6	253.7	248.1	226.3	306.1	370	
372	330.4	173.7	178.8	177.9	171.6	255.1	249.5	227.6	307.8	372	
374	332.2	174.7	179.8	179.0	172.7	256.5	250.9	228.9	309.5	374	
376	334.0	175.8	180.9	180.0	173.7	257.9	252.2	230.2	311.1	376	
378	335.8	176.8	182.0	181.1	174.8	259.3	253.6	231.5	312.8	378	
380	337.5	177.9	183.0	182.1	175.9	260.7	255.0	232.8	314.5	380	
382	339.3	178.9	184.1	183.2	176.9	262.1	256.4	234.1	316.1	382	
384	341.1	180.0	185.2	184.3	178.0	263.5	257.8	235.4	317.8	384	
386	342.9	181.0	186.2	185.4	179.1	264.9	259.2	236.6	319.4	386	
388	344.6	182.0	187.3	186.4	180.1	266.5	260.5	237.9	321.1	388	
390	346.4	183.1	188.4	187.5	181.2	267.7	261.9	239.2	322.8	390	
392	348.2	184.1	189.4	188.6	182.3	269.1	263.3	240.5	324.4	392	
394	350.0	185.2	190.5	189.7	183.3	270.5	264.7	241.8	326.1	394	
396	351.8	186.2	191.6	190.7	184.4	271.9	266.1	243.1	327.7	396	
398	353.5	187.3	192.7	191.8	185.5	273.3	267.5	244.4	329.4	398	
400	355.3	188.4	193.7	192.9	186.5	274.7	268.9	245.7	331.1	400	
402	357.1	189.4	194.8	194.0	187.6	276.1	270.3	247.0	332.7	402	
404	358.9	190.5	195.9	195.0	188.7	277.5	271.7	248.3	334.4	404	
406	360.6	191.5	197.0	196.1	189.8	278.9	273.0	249.6	336.0	406	
408	362.4	192.6	198.1	197.2	190.8	280.3	274.4	251.0	337.7	408	

*Munson and Walker's table.*—Concluded.  
(Expressed in milligrams.)

9

CUPROUS OXIDE (Cu <sub>2</sub> O)	COPPER (Cu)	DEXTRINE (d-aldose)	INVERT SUGAR	INVERT SUGAR AND SUCROSE		LACTOSE C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> +H <sub>2</sub> O	LACTOSE AND SUCROSE		MALTOSE C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> +H <sub>2</sub> O	CUPROUS OXIDE (Cu <sub>2</sub> O)
				0.4 gram total sugar	2 grams total sugar		1 lactose, 4 sucrose	1 lactose, 12 sucrose		
410	364.2	193.7	199.1	198.3	191.9	281.7	275.8	252.3	339.4	410
412	366.0	194.7	200.2	199.4	193.0	283.2	277.2	253.6	341.0	412
414	367.7	195.8	201.3	200.5	194.1	284.6	278.6	254.9	342.7	414
416	369.5	196.8	202.4	201.6	195.2	286.0	280.0	256.2	344.4	416
418	371.3	197.9	203.5	202.6	196.2	287.4	281.4	257.5	346.0	418
420	373.1	199.0	204.6	203.7	197.3	288.8	282.8	258.8	347.7	420
422	374.8	200.1	205.7	204.8	198.4	290.2	284.2	260.1	349.3	422
424	376.6	201.1	206.7	205.9	199.5	291.6	285.6	261.4	351.0	424
426	378.4	202.2	207.8	207.0	200.6	293.0	287.0	262.7	352.7	426
428	380.2	203.3	208.9	208.1	201.7	294.4	288.4	264.0	354.3	428
430	382.0	204.4	210.0	209.2	202.7	295.8	289.8	265.4	356.0	430
432	383.7	205.5	211.1	210.3	203.8	297.2	291.2	266.6	357.6	432
434	385.5	206.5	212.2	211.4	204.9	298.6	292.6	268.0	359.3	434
436	387.3	207.6	213.3	212.5	206.0	300.0	294.0	269.3	361.0	436
438	389.1	208.7	214.4	213.6	207.1	301.4	295.4	270.6	362.6	438
440	390.8	209.8	215.5	214.7	208.2	302.8	296.8	272.0	364.3	440
442	392.6	210.9	216.6	215.8	209.3	304.2	298.2	273.3	365.9	442
444	394.4	212.0	217.8	216.9	210.4	305.6	299.6	274.6	367.6	444
446	396.2	213.1	218.9	218.0	211.5	307.0	301.0	275.9	369.3	446
448	397.9	214.1	220.0	219.1	212.6	308.4	302.4	277.2	370.9	448
450	399.7	215.2	221.1	220.2	213.7	309.9	303.8	278.6	372.6	450
452	401.5	216.3	222.2	221.4	214.8	311.3	305.2	279.9	374.2	452
454	403.3	217.4	223.3	222.5	215.9	312.7	306.6	281.2	375.9	454
456	405.1	218.5	224.4	223.6	217.0	314.1	308.0	282.5	377.6	456
458	406.8	219.6	225.5	224.7	218.1	315.5	309.4	283.9	379.2	458
460	408.6	220.7	226.7	225.8	219.2	316.9	310.8	285.2	380.9	460
462	410.4	221.8	227.8	226.9	220.3	318.3	312.2	286.5	382.5	462
464	412.2	222.9	228.9	228.1	221.4	319.7	313.6	287.8	384.2	464
466	413.9	224.0	230.0	229.2	222.5	321.1	315.0	289.2	385.9	466
468	415.7	225.1	231.2	230.3	223.7	322.5	316.4	290.5	387.5	468
470	417.5	226.2	232.3	231.4	224.8	323.9	317.7	291.8	389.2	470
472	419.3	227.4	233.4	232.5	225.9	325.3	319.1	293.2	390.8	472
474	421.0	228.3	234.5	233.7	227.0	326.8	320.5	294.5	392.5	474
476	422.8	229.6	235.7	234.8	228.1	328.2	321.9	295.8	394.2	476
478	424.6	230.7	236.8	235.9	229.2	329.6	323.3	297.1	395.8	478
480	426.4	231.8	237.9	237.1	230.3	331.0	324.7	298.5	397.5	480
482	428.1	232.9	239.1	238.2	231.5	332.4	326.1	299.8	399.1	482
484	429.9	234.1	240.2	239.3	232.6	333.8	327.5	301.1	400.8	484
486	431.7	235.2	241.4	240.5	233.7	335.2	328.9	302.5	402.4	486
488	433.5	236.3	242.5	241.6	234.8	336.6	330.3	303.8	404.1	488
490	435.3	237.4	243.6	242.7	236.0	338.0	331.7	305.1	405.8	490

10 *Herzfeld's table for determining invert sugar in raw sugars (invert sugar not to exceed 1.5 per cent).<sup>1</sup>*

COPPER (Cu)	INVERT SUGAR	COPPER (Cu)	INVERT SUGAR	COPPER (Cu)	INVERT SUGAR	COPPER (Cu)	INVERT SUGAR	COPPER (Cu)	INVERT SUGAR
mg.	per cent	mg.	per cent	mg.	per cent	mg.	per cent	mg.	per cent
50	0.050	110	0.351	170	0.680	230	1.013	290	1.357
52	.058	112	.361	172	.692	232	.024	292	.368
54	.066	114	.371	174	.704	234	.036	294	.380
56	.074	116	.381	176	.715	236	.047	296	.391
58	.082	118	.392	178	.726	238	.058	298	.403
60	.090	120	.402	180	.737	240	.070	300	.414
62	.098	122	.412	182	.748	242	.081	302	.425
64	.108	124	.423	184	.759	244	.093	304	.437
66	.118	126	.433	186	.770	246	.104	306	.448
68	.128	128	.443	188	.781	248	.116	308	.460
70	.138	130	.453	190	.792	250	.127	310	.471
72	.148	132	.463	192	.803	252	.139	312	.483
74	.157	134	.473	194	.814	254	.150	314	.494
76	.167	136	.483	196	.825	256	.162		
78	.177	138	.493	198	.836	258	.173		
80	.187	140	.503	200	.847	260	.185		
82	.197	142	.515	202	.858	262	.196		
84	.208	144	.527	204	.869	264	.207		
86	.219	146	.538	206	.880	266	.219		
88	.231	148	.550	208	.891	268	.231		
90	.242	150	.562	210	.902	270	.242		
92	.254	152	.574	212	.913	272	.253		
94	.265	154	.586	214	.924	274	.265		
96	.277	156	.598	216	.935	276	.276		
98	.288	158	.609	218	.946	278	.288		
100	.300	160	.621	220	.957	280	.299		
102	.310	162	.633	222	.968	282	.311		
104	.320	164	.645	224	.979	284	.322		
106	.330	166	.657	226	.990	286	.334		
108	.340	168	.669	228	1.001	288	.345		

<sup>1</sup> *Z. Ver. Ruckenzucker-Ind.*, 35 (N.F. 22); 1012 (1885).

*Meissi and Hiller's factors for determining invert sugar in materials in which, of the total sugars present, more than 1.5 per cent is invert sugar, and less than 98.5 per cent is sucrose.<sup>1</sup>*

RATIO OF SUCROSE TO INVERT SUGAR = R:1	APPROXIMATE ABSOLUTE WEIGHT OF INVERT SUGAR (Z)						
	200 mg	175 mg	150 mg	125 mg	100 mg	75 mg	50 mg
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
0:100	56.4	55.4	54.5	53.8	53.2	53.0	53.0
10:90	56.3	55.3	54.4	53.8	53.2	52.9	52.9
20:80	56.2	55.2	54.3	53.7	53.2	52.7	52.7
30:70	56.1	55.1	54.2	53.7	53.2	52.6	52.6
40:60	55.9	55.0	54.1	53.6	53.1	52.5	52.4
50:50	55.7	54.9	54.0	53.5	53.1	52.3	52.2
60:40	55.6	54.7	53.8	53.2	52.8	52.1	51.9
70:30	55.5	54.5	53.5	52.9	52.5	51.9	51.6
80:20	55.4	54.3	53.3	52.7	52.2	51.7	51.3
90:10	54.6	53.6	53.1	52.6	52.1	51.6	51.2
91:9	54.1	53.6	52.6	52.1	51.6	51.2	50.7
92:8	53.6	53.1	52.1	51.6	51.2	50.7	50.3
93:7	53.6	53.1	52.1	51.2	50.7	50.3	49.8
94:6	53.1	52.6	51.6	50.7	50.3	49.8	48.9
95:5	52.6	52.1	51.2	50.3	49.4	48.9	48.5
96:4	52.1	51.2	50.7	49.8	48.9	47.7	46.9
97:3	50.7	50.3	49.8	48.9	47.7	46.2	45.1
98:2	49.9	48.9	48.5	47.3	45.8	43.3	40.0
99:1	47.7	47.3	46.5	45.1	43.3	41.2	38.1

<sup>1</sup> Z. Ver. Ruchenzucker-Ind., 39 (N.F. 26), 734, (1889).

Wein's table for the determination of maltose.<sup>1</sup>  
(Expressed in milligrams.)

COPPER	CUPROUS OXIDE	MALTOSE	COPPER	CUPROUS OXIDE	MALTOSE	COPPER	CUPROUS OXIDE	MALTOSE
32	36.0	27.0	122	137.4	106.2	212	238.7	186.8
34	38.3	28.7	124	139.6	108.0	214	240.9	188.6
36	40.5	30.5	126	141.9	109.8	216	243.2	190.4
38	42.8	32.2	128	144.1	111.6	218	245.4	192.1
40	45.0	33.9	130	146.4	113.4	220	247.7	193.9
42	47.3	35.7	132	148.6	115.2	222	249.9	195.7
44	49.5	37.4	134	150.9	117.0	224	252.4	197.5
46	51.8	39.1	136	153.1	118.8	226	254.4	199.3
48	54.0	40.9	138	155.4	120.6	228	256.7	201.1
50	56.3	42.6	140	157.6	122.4	230	258.9	202.9
52	58.5	44.4	142	159.9	124.2	232	261.2	204.7
54	60.8	46.1	144	162.1	126.0	234	263.4	206.5
56	63.0	47.8	146	164.4	127.8	236	265.7	208.3
58	65.3	49.6	148	166.6	129.6	238	268.0	210.0
60	67.6	51.3	150	168.9	131.4	240	270.2	211.8
62	69.8	53.1	152	171.1	133.2	242	272.5	213.6
64	72.1	54.8	154	173.4	135.0	244	274.7	215.4
66	74.3	56.6	156	175.6	136.8	246	277.0	217.2
68	76.6	58.3	158	177.9	138.6	248	279.2	219.0
70	78.8	60.1	160	180.1	140.4	250	281.5	220.8
72	81.1	61.8	162	182.4	142.2	252	283.7	222.6
74	83.3	63.6	164	184.6	144.0	254	286.0	224.4
76	85.6	65.4	166	186.9	145.8	256	288.2	226.2
78	87.8	67.1	168	189.1	147.6	258	290.5	228.0
80	90.1	68.9	170	191.4	149.4	260	292.7	229.8
82	92.3	70.6	172	193.6	151.2	262	295.0	231.6
84	94.6	72.4	174	195.9	152.9	264	297.2	233.4
86	96.8	74.1	176	198.1	154.7	266	299.5	235.2
88	99.1	75.9	178	200.4	156.5	268	301.7	237.0
90	101.3	77.7	180	202.6	158.3	270	304.0	238.8
92	103.6	79.5	182	204.9	160.1	272	306.2	240.6
94	105.8	81.2	184	207.1	161.8	274	308.5	242.4
96	108.1	83.0	186	209.4	163.6	276	310.7	244.2
98	110.3	84.8	188	211.7	165.4	278	313.0	246.0
100	112.6	86.6	190	213.9	167.2	280	315.2	247.8
102	114.8	88.4	192	216.2	169.0	282	317.5	249.6
104	117.1	90.1	194	218.4	170.7	284	319.7	251.3
106	119.3	91.9	196	220.7	172.5	286	322.0	253.1
108	121.6	93.7	198	222.9	174.3	288	324.2	254.9
110	123.8	95.5	200	225.2	176.1	290	326.5	256.6
112	126.1	97.3	202	227.4	177.9	292	328.7	258.4
114	128.3	99.0	204	229.7	179.6	294	331.0	260.2
116	130.6	100.8	206	231.9	181.4	296	333.2	262.0
118	132.8	102.6	208	234.2	183.2	298	335.5	263.7
120	135.1	104.4	210	236.4	185.0	300	337.8	265.5

<sup>1</sup> Tables for the Quantitative Estimation of the Sugars. Translated by Frew, 1896, p. 26.

*Copper-levulose equivalents according to Jackson and Mathews' modification of Nyns' selective method for levulose.*

(Expressed in milligrams. A linear interpolation yields accurate results.)

Cu	LEVULOSE	Cu	LEVULOSE
5	2.5	130	39.3
10	4.5	140	42.0
15	6.2	150	44.7
20	7.9	160	47.4
25	9.5	170	50.0
30	11.0	180	52.6
35	12.5	190	55.2
40	13.9	200	57.9
45	15.4	210	60.6
50	16.8	220	63.4
55	18.3	230	66.4
60	19.7	240	69.4
65	21.2	250	72.5
70	22.5	260	75.7
80	25.4	270	79.0
90	28.1	280	82.4
100	30.9	290	85.9
110	33.7	300	89.5
120	36.5	310	93.2

*Allihn's table for the determination of dextrose.<sup>1</sup>*  
(Expressed in milligrams.)

COFFER	CUPROUS OXIDE	DEXTROSE	COFFER	CUPROUS OXIDE	DEXTROSE	COFFER	CUPROUS OXIDE	DEXTROSE
12	13.5	7.1	102	114.8	51.9	192	216.2	98.4
14	15.8	8.1	104	117.1	52.9	194	218.4	99.4
16	18.0	9.0	106	119.3	54.0	196	220.7	100.5
18	20.3	10.0	108	121.6	55.0	198	222.9	101.5
20	22.5	11.0	110	123.8	56.0	200	225.2	102.6
22	24.8	12.0	112	126.1	57.0	202	227.4	103.7
24	27.0	13.0	114	128.3	58.0	204	229.7	104.7
26	29.3	14.0	116	130.6	59.1	206	231.9	105.8
28	31.5	15.0	118	132.8	60.1	208	234.2	106.8
30	33.8	16.0	120	135.1	61.1	210	236.4	107.9
32	36.0	17.0	122	137.4	62.1	212	238.7	109.0
34	38.3	18.0	124	139.6	63.1	214	240.9	110.0
36	40.5	18.9	126	141.9	64.2	216	243.2	111.1
38	42.8	19.9	128	144.1	65.2	218	245.4	112.1
40	45.0	20.9	130	146.4	66.2	220	247.7	113.2
42	47.3	21.9	132	148.6	67.2	222	249.9	114.3
44	49.5	22.9	134	150.9	68.2	224	252.4	115.3
46	51.8	23.9	136	153.1	69.3	226	254.4	116.4
48	54.0	24.9	138	155.4	70.3	228	256.7	117.4
50	56.3	25.9	140	157.6	71.3	230	258.9	118.5
52	58.5	26.9	142	159.9	72.3	232	261.2	119.6
54	60.8	27.9	144	162.1	73.4	234	263.4	120.7
56	63.0	28.8	146	164.4	74.4	236	265.7	121.7
58	65.3	29.8	148	166.6	75.5	238	268.0	122.8
60	67.6	30.8	150	168.9	76.5	240	270.2	123.9
62	69.8	31.8	152	171.1	77.5	242	272.5	125.0
64	72.1	32.8	154	173.4	78.6	244	274.7	126.0
66	74.3	33.8	156	175.6	79.6	246	277.0	127.1
68	76.6	34.8	158	177.9	80.7	248	279.2	128.1
70	78.8	35.8	160	180.1	81.7	250	281.5	129.2
72	81.1	36.8	162	182.4	82.6	252	283.7	130.3
74	83.3	37.8	164	184.6	83.7	254	286.0	131.4
76	85.6	38.8	166	186.9	84.8	256	288.2	132.4
78	87.8	39.8	168	189.1	85.9	258	290.5	133.5
80	90.1	40.8	170	191.4	86.9	260	292.7	134.6
82	92.3	41.8	172	193.6	87.9	262	295.0	135.7
84	94.6	42.8	174	195.9	89.0	264	297.2	136.8
86	96.8	43.9	176	198.1	90.0	266	299.5	137.8
88	99.1	44.9	178	200.4	91.1	268	301.7	138.9
90	101.3	45.9	180	202.6	92.1	270	304.0	140.0
92	103.6	46.9	182	204.9	93.1	272	306.2	141.1
94	105.8	47.9	184	207.1	94.2	274	308.5	142.2
96	108.1	48.9	186	209.4	95.2	276	310.7	143.3
98	110.3	49.9	188	211.7	96.3	278	313.0	144.4
100	112.6	50.9	190	213.9	97.3	280	315.2	145.5

<sup>1</sup> *Z. Ver. Ruohenzucker-Ind.*, 32 (N.F. 19), 606, 865 (1882).

*Allihn's table.*—Concluded.

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(Expressed in milligrams.)

COPPER	CUPROUS OXIDE	DEXTRÖSE	COPPER	CUPROUS OXIDE	DEXTRÖSE	COPPER	CUPROUS OXIDE	DEXTRÖSE
282	317.5	146.6	342	385.0	179.8	402	452.6	214.1
284	319.7	147.7	344	387.3	180.9	404	454.8	215.2
286	322.0	148.8	346	389.6	182.1	406	457.1	216.4
288	324.2	149.9	348	391.8	183.2	408	459.4	217.5
290	326.5	151.0	350	394.0	184.3	410	461.6	218.7
292	328.7	152.1	352	396.3	185.4	412	463.8	219.9
294	331.0	153.2	354	398.6	186.6	414	466.1	221.0
296	333.3	154.3	356	400.8	187.7	416	468.4	222.2
298	335.5	155.4	358	403.1	188.9	418	470.6	223.3
300	337.8	156.5	360	405.3	190.0	420	472.9	224.5
302	340.0	157.6	362	407.6	191.1	422	475.6	225.7
304	342.3	158.7	364	409.8	192.3	424	477.4	226.9
306	344.5	159.8	366	412.1	193.4	426	479.6	228.0
308	346.8	160.9	368	414.3	194.6	428	481.9	229.2
310	349.0	162.0	370	416.6	195.7	430	484.1	230.4
312	351.3	163.1	372	418.8	196.8	432	486.4	231.6
314	353.5	164.2	374	421.1	198.0	434	488.6	232.8
316	355.8	165.3	376	423.3	199.1	436	490.9	233.9
318	358.0	166.4	378	425.6	200.3	438	493.1	235.1
320	360.3	167.5	380	427.8	201.4	440	495.4	236.3
322	362.5	168.6	382	430.1	202.5	442	497.6	237.5
324	364.8	169.7	384	432.3	203.7	444	499.9	238.7
326	367.0	170.9	386	434.6	204.8	446	502.1	239.8
328	369.3	172.0	388	436.8	206.0	448	504.4	241.0
330	371.5	173.1	390	439.1	207.1	450	506.6	242.2
332	373.8	174.2	392	441.3	208.3	452	508.9	243.4
334	376.0	175.3	394	443.6	209.4	454	511.1	244.6
336	378.3	176.5	396	445.9	210.6	456	513.4	245.7
338	380.5	177.6	398	448.1	211.7	458	515.6	246.9
340	382.8	178.7	400	450.3	212.9	460	517.9	248.1
						462	520.1	249.3



15 Factors for 10 cc Sozhet's solution to be used in connection with the Lane-Eynon general volumetric method.

TITER	NO SUCROSE INVERT SUGAR	1 GRAM SUCROSE PER 100 CC INVERT SUGAR	5 GRAMS SUCROSE PER 100 CC INVERT SUGAR	10 GRAMS SUCROSE PER 100 CC INVERT SUGAR	25 GRAMS SUCROSE PER 100 CC INVERT SUGAR	DEXTROROSE	LEVULOSE	ANHYDROUS MALTOSE $C_{12}H_{22}O_{11}$	HYDRATED MALTOSE $C_{12}H_{22}O_{11} \cdot H_2O$	ANHYDROUS LACTOSE $C_{12}H_{22}O_{11}$	HYDRATED LACTOSE $C_{12}H_{22}O_{11} \cdot H_2O$
15	50.5	49.9	47.6	46.1	43.4	49.1	52.2	77.2	81.3	64.9	68.3
16	50.6	50.0	47.6	46.1	43.4	49.2	52.3	77.1	81.2	64.8	68.2
17	50.7	50.1	47.6	46.1	43.4	49.3	52.3	77.0	81.1	64.8	68.2
18	50.8	50.1	47.6	46.1	43.3	49.3	52.4	77.0	81.0	64.7	58.1
19	50.8	50.2	47.6	46.1	43.3	49.4	52.5	76.9	80.9	64.7	68.1
20	50.9	50.2	47.6	46.1	43.2	49.5	52.5	76.8	80.8	64.6	68.0
21	51.0	50.2	47.6	46.1	43.2	49.5	52.6	76.7	80.7	64.6	68.0
22	51.0	50.3	47.6	46.1	43.1	49.6	52.7	76.6	80.6	64.6	68.0
23	51.1	50.3	47.6	46.1	43.0	49.7	52.7	76.5	80.5	64.5	67.9
24	51.2	50.3	47.6	46.1	42.9	49.8	52.8	76.4	80.4	64.5	67.9
25	51.2	50.4	47.6	46.0	42.8	49.8	52.8	76.4	80.4	64.5	67.9
26	51.3	50.4	47.6	46.0	42.8	49.9	52.9	76.3	80.3	64.5	67.9
27	51.4	50.4	47.6	46.0	42.7	49.9	52.9	76.2	80.2	64.4	67.8
28	51.4	50.5	47.7	46.0	42.7	50.0	53.0	76.1	80.1	64.4	67.8
29	51.5	50.5	47.7	46.0	42.6	50.0	53.1	76.0	80.0	64.4	67.8
30	51.5	50.5	47.7	46.0	42.5	50.1	53.2	76.0	80.0	64.4	67.8
31	51.6	50.6	47.7	45.9	42.5	50.2	53.2	75.9	79.9	64.4	67.8
32	51.6	50.6	47.7	45.9	42.4	50.2	53.3	75.9	79.9	64.4	67.8
33	51.7	50.6	47.7	45.9	42.3	50.3	53.3	75.8	79.8	64.4	67.8
34	51.7	50.6	47.7	45.8	42.2	50.3	53.4	75.8	79.8	64.4	67.9
35	51.8	50.7	47.7	45.8	42.2	50.4	53.4	75.7	79.7	64.5	67.9
36	51.8	50.7	47.7	45.8	42.1	50.4	53.5	75.6	79.6	64.5	67.9
37	51.9	50.7	47.7	45.7	42.0	50.5	53.5	75.6	79.6	64.5	67.9
38	51.9	50.7	47.7	45.7	42.0	50.5	53.6	75.5	79.5	64.5	67.9
39	52.0	50.8	47.7	45.7	41.9	50.6	53.6	75.5	79.5	64.5	67.9
40	52.0	50.8	47.7	45.6	41.8	50.6	53.6	75.4	79.4	64.5	67.9
41	52.1	50.8	47.7	45.6	41.8	50.7	53.7	75.4	79.4	64.6	68.0
42	52.1	50.8	47.7	45.6	41.7	50.7	53.7	75.3	79.3	64.6	68.0
43	52.2	50.8	47.7	45.5	41.6	50.8	53.8	75.3	79.3	64.6	68.0
44	52.2	50.9	47.7	45.5	41.5	50.8	53.8	75.2	79.2	64.6	68.0
45	52.3	50.9	47.7	45.4	41.4	50.9	53.9	75.2	79.2	64.7	68.1
46	52.3	50.9	47.7	45.4	41.4	50.9	53.9	75.1	79.1	64.7	68.1
47	52.4	50.9	47.7	45.3	41.3	51.0	53.9	75.1	79.1	64.8	68.2
48	52.4	50.9	47.7	45.3	41.2	51.0	54.0	75.1	79.1	64.8	68.2
49	52.5	51.0	47.7	45.2	41.1	51.0	54.0	75.0	79.0	64.8	68.2
50	52.5	51.0	47.7	45.2	41.0	51.1	54.0	75.0	79.0	64.9	68.3

## REFERENCE TABLES

XLII

Factors for 25 cc Sozhiel's solution to be used in connection with the  
Lane-Eynon general volumetric method.

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TITER	NO SUCROSE INVERT SUGAR	1 GRAM SUCROSE PER 100 CC INVERT SUGAR	DEXTROSE	LEVULOSE	ANHYDROUS MALTOSE $C_{12}H_{22}O_{11}$	HYDRATED MALTOSE $C_{12}H_{22}O_{11} \cdot H_2O$	ANHYDROUS LACTOSE $C_{12}H_{22}O_{11}$	HYDRATED LACTOSE $C_{12}H_{22}O_{11} \cdot H_2O$
15	123.6	122.6	120.2	127.4	197.8	208.2	163.9	172.5
16	123.6	122.7	120.2	127.4	197.4	207.8	163.5	172.1
17	123.6	122.7	120.2	127.5	197.0	207.4	163.1	171.7
18	123.7	122.7	120.2	127.5	196.7	207.1	162.8	171.4
19	123.7	122.8	120.3	127.6	196.5	206.8	162.5	171.1
20	123.8	122.8	120.3	127.6	196.2	206.5	162.3	170.9
21	123.8	122.8	120.3	127.7	195.8	206.1	162.0	170.6
22	123.9	122.9	120.4	127.7	195.5	205.8	161.8	170.4
23	123.9	122.9	120.4	127.8	195.1	205.4	161.6	170.2
24	124.0	122.9	120.5	127.8	194.8	205.1	161.5	170.0
25	124.0	123.0	120.5	127.9	194.5	204.8	161.4	169.9
26	124.1	123.0	120.6	127.9	194.2	204.4	161.2	169.7
27	124.1	123.0	120.6	128.0	193.9	204.1	161.0	169.5
28	124.2	123.1	120.7	128.0	193.6	203.8	160.8	169.3
29	124.2	123.1	120.7	128.1	193.3	203.5	160.7	169.2
30	124.3	123.1	120.8	128.1	193.0	203.2	160.6	169.0
31	124.3	123.2	120.8	128.1	192.8	202.9	160.5	168.9
32	124.4	123.2	120.8	128.2	192.5	202.6	160.4	168.8
33	124.4	123.2	120.9	128.2	192.2	202.3	160.2	168.6
34	124.5	123.3	120.9	128.3	191.9	202.0	160.1	168.5
35	124.5	123.3	121.0	128.3	191.7	201.8	160.0	168.4
36	124.6	123.3	121.0	128.4	191.4	201.5	159.8	168.2
37	124.6	123.4	121.1	128.4	191.2	201.2	159.7	168.1
38	124.7	123.4	121.2	128.5	191.0	201.0	159.6	168.0
39	124.7	123.4	121.2	128.5	190.8	200.8	159.5	167.9
40	124.8	123.4	121.2	128.6	190.5	200.5	159.4	167.8
41	124.8	123.5	121.3	128.6	190.3	200.3	159.3	167.7
42	124.9	123.5	121.4	128.6	190.1	200.1	159.2	167.6
43	124.9	123.5	121.4	128.7	189.8	199.8	159.2	167.6
44	125.0	123.6	121.5	128.7	189.6	199.6	159.1	167.5
45	125.0	123.6	121.5	128.8	189.4	199.4	159.0	167.4
46	125.1	123.6	121.6	128.8	189.2	199.2	159.0	167.4
47	125.1	123.7	121.6	128.9	189.0	199.0	158.9	167.3
48	125.2	123.7	121.7	128.9	188.9	198.9	158.8	167.2
49	125.2	123.7	121.7	129.0	188.8	198.7	158.8	167.2
50	125.3	123.8	121.8	129.0	188.7	198.6	158.7	167.1

17 *Quisumbing and Thomas table for calculating dextrose, levulose, invert sugar, lactose, and maltose.*  
(Expressed in milligrams.)

COPPER (Cu)	CUPROSE OXIDE (Cu <sub>2</sub> O)	DEXTROSE (D-GLUCOSE)	LEVULOSE (D-FRUCTOSE)	INVERT SUGAR	LACTOSE		MALTOSE	
					C <sub>12</sub> H <sub>22</sub> O <sub>11</sub>	C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> · H <sub>2</sub> O	C <sub>12</sub> H <sub>22</sub> O <sub>11</sub>	C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> · H <sub>2</sub> O
10	11.1	4.8	5.3	5.0	7.7	8.1	9.4	9.9
20	22.5	9.5	10.5	10.1	15.5	16.3	18.8	19.8
30	33.8	14.3	15.8	15.2	23.2	24.4	28.2	29.7
40	45.0	19.1	21.2	20.3	30.9	32.5	37.6	39.6
50	56.3	24.0	26.5	25.4	38.7	40.7	47.0	49.5
60	67.6	28.9	31.9	30.6	46.4	48.8	56.4	59.4
70	78.8	33.7	37.2	35.7	54.0	56.9	65.8	69.3
80	90.1	38.7	42.6	40.9	61.7	65.9	75.2	79.2
90	101.3	43.6	48.0	46.1	69.5	73.2	84.6	89.1
100	112.6	48.6	53.4	51.3	77.2	81.3	94.0	99.0
110	123.8	53.5	58.8	56.5	85.0	89.5	103.4	108.9
120	135.1	58.5	64.3	61.8	92.7	97.0	112.8	118.8
130	146.4	63.5	70.7	67.0	100.4	105.7	122.2	128.7
140	157.6	68.6	75.2	72.3	108.2	113.9	131.6	138.6
150	168.9	73.7	80.7	77.6	116.0	122.0	141.0	148.5
160	180.1	78.8	86.2	82.9	123.7	130.1	150.4	158.4
170	191.4	83.9	91.7	88.3	131.4	138.3	159.8	168.3
180	202.6	89.1	97.2	93.7	139.1	146.4	169.2	178.2
190	213.9	94.2	102.8	99.1	146.9	154.6	178.8	188.1
200	225.2	99.4	108.4	104.4	154.6	162.7	188.2	198.0
210	236.4	104.6	114.0	109.8	162.3	170.9	197.6	207.9
220	247.7	109.9	119.6	115.2	170.0	179.0	207.0	217.8
230	258.9	115.1	125.2	120.6	177.8	187.2	216.4	227.7
240	270.2	120.4	130.8	126.1	185.5	195.3	225.8	237.6
250	281.5	125.7	136.4	131.6	193.2	203.4	235.2	247.5
260	292.7	131.0	142.1	137.1	201.0	211.6	244.6	257.4
270	304.0	136.4	147.8	142.6	208.8	219.8	254.0	267.3
280	315.2	141.7	153.5	148.2	216.5	227.9	263.4	277.2
290	326.5	147.1	159.2	153.7	224.2	236.0	272.8	287.1
300	337.8	152.6	165.0	159.3	232.0	244.2	282.2	297.0
310	349.0	158.0	170.7	164.9	239.7	252.3	291.6	306.9
320	360.3	163.5	176.5	170.5	247.5	260.5	301.0	316.8
330	371.5	168.9	182.3	176.1	255.3	268.7	310.4	326.7
340	382.8	174.5	188.1	181.8	263.0	276.8	319.8	336.6
350	394.0	180.0	193.9	187.4	270.7	285.0	329.2	346.5
360	405.3	185.5	199.7	193.1	278.4	293.1	338.6	356.4
370	416.6	191.1	205.5	198.8	286.2	301.3	348.0	366.3
380	427.8	196.7	211.4	204.5	293.9	309.4	357.4	376.2
390	439.1	202.3	217.3	210.2	301.6	317.5	366.8	386.1
400	450.3	208.0	223.2	216.0	309.4	325.7	376.2	396.0
410	461.6	213.7	229.1	221.8	317.1	333.8	385.6	405.9
420	472.9	219.4	235.0	227.6	324.9	342.0	395.0	415.8
430	484.1	225.1	240.9	233.4	332.6	350.1	404.4	425.7
440	495.4	230.8	246.9	239.2	340.4	358.3	413.8	435.6
450	506.6	236.6	252.9	245.0	348.1	366.4	423.2	445.5
460	517.9	242.4	258.9	250.9	355.9	374.6	432.6	455.4
470	529.1	248.1	264.9	256.8	363.6	382.7	442.0	465.3
480	540.4	250.8	270.9	262.7	371.3	390.9	451.4	475.2

*Kröber's table for the determination of pentoses and pentosans.*  
(Expressed in grams.)

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FURFURAL PHLOROLUCIDE	FURFURAL	ARABINOSE	ARABAN	XYLOSE	XYLAN	PENTOSE	PENTOSAN
0.030	0.0182	0.0391	0.0344	0.0324	0.0285	0.0358	0.0315
.032	.0193	.0413	.0363	.0342	.0301	.0378	.0333
.034	.0203	.0435	.0383	.0361	.0317	.0398	.0350
.036	.0214	.0457	.0402	.0379	.0334	.0418	.0368
.038	.0224	.0479	.0422	.0398	.0350	.0439	.0386
.040	.0235	.0501	.0441	.0416	.0366	.0459	.0404
.042	.0245	.0523	.0460	.0434	.0382	.0479	.0422
.044	.0255	.0545	.0480	.0452	.0398	.0499	.0440
.046	.0266	.0567	.0499	.0471	.0414	.0519	.0457
.048	.0276	.0589	.0519	.0489	.0430	.0539	.0475
.050	.0286	.0611	.0538	.0507	.0446	.0559	.0492
.052	.0297	.0633	.0557	.0525	.0462	.0579	.0510
.054	.0307	.0655	.0576	.0543	.0478	.0599	.0528
.056	.0318	.0677	.0596	.0562	.0494	.0620	.0546
.058	.0328	.0699	.0615	.0580	.0510	.0640	.0564
.060	.0338	.0721	.0634	.0598	.0526	.0660	.0581
.062	.0349	.0743	.0653	.0616	.0542	.0680	.0599
.064	.0359	.0765	.0673	.0635	.0558	.0700	.0617
.066	.0370	.0787	.0692	.0653	.0575	.0720	.0634
.068	.0380	.0809	.0712	.0672	.0591	.0741	.0652
.070	.0390	.0831	.0731	.0690	.0607	.0761	.0670
.072	.0401	.0853	.0750	.0708	.0623	.0781	.0688
.074	.0411	.0875	.0770	.0726	.0639	.0801	.0706
.076	.0422	.0897	.0789	.0745	.0655	.0821	.0722
.078	.0432	.0919	.0809	.0763	.0671	.0841	.0740
.080	.0442	.0941	.0828	.0781	.0687	.0861	.0758
.082	.0453	.0963	.0847	.0799	.0703	.0881	.0776
.084	.0463	.0985	.0867	.0817	.0719	.0901	.0794
.086	.0474	.1007	.0886	.0836	.0735	.0922	.0812
.088	.0484	.1029	.0906	.0854	.0751	.0942	.0830
.090	.0494	.1051	.0925	.0872	.0767	.0962	.0847
.092	.0505	.1073	.0944	.0890	.0783	.0982	.0865
.094	.0515	.1095	.0964	.0909	.0800	.1002	.0883
.096	.0525	.1117	.0983	.0927	.0816	.1022	.0899
.098	.0536	.1139	.1003	.0946	.0832	.1043	.0917
.100	.0546	.1161	.1022	.0964	.0848	.1063	.0935
.102	.0557	.1182	.1041	.0982	.0864	.1083	.0953
.104	.0567	.1204	.1060	.1000	.0880	.1103	.0971
.106	.0577	.1226	.1080	.1019	.0896	.1123	.0988
.108	.0588	.1248	.1099	.1037	.0912	.1143	.1006
.110	.0598	.1270	.1118	.1055	.0928	.1163	.1023
.112	.0608	.1292	.1137	.1073	.0944	.1183	.1041
.114	.0619	.1314	.1156	.1091	.0960	.1203	.1059
.116	.0629	.1336	.1176	.1110	.0976	.1223	.1076
.118	.0640	.1358	.1195	.1128	.0992	.1243	.1094

FURFURAL PHLOEOGLUCIDE	FURFURAL	ARABINOSE	ARABAN	XYLOSE	XYLAN	PENTOSE	PENTOBAN
0.120	0.0650	0.1380	0.1214	0.1146	0.1008	0.1263	0.1111
.122	.0660	.1402	.1233	.1164	.1024	.1283	.1129
.124	.0671	.1424	.1253	.1182	.1040	.1303	.1147
.126	.0681	.1446	.1272	.1201	.1057	.1324	.1165
.128	.0691	.1468	.1292	.1219	.1073	.1344	.1183
.130	.0702	.1490	.1311	.1237	.1089	.1364	.1201
.132	.0712	.1512	.1330	.1255	.1105	.1384	.1219
.134	.0723	.1534	.1350	.1273	.1121	.1404	.1236
.136	.0733	.1556	.1369	.1292	.1137	.1424	.1253
.138	.0743	.1578	.1389	.1310	.1153	.1444	.1271
.140	.0754	.1600	.1408	.1328	.1169	.1464	.1288
.142	.0764	.1622	.1427	.1346	.1185	.1484	.1306
.144	.0774	.1644	.1447	.1364	.1201	.1504	.1324
.146	.0785	.1666	.1466	.1383	.1217	.1525	.1342
.148	.0795	.1688	.1486	.1401	.1233	.1545	.1360
.150	.0805	.1710	.1505	.1419	.1249	.1565	.1377
.152	.0816	.1732	.1524	.1437	.1265	.1585	.1395
.154	.0826	.1754	.1544	.1455	.1281	.1605	.1413
.156	.0837	.1776	.1563	.1474	.1297	.1625	.1430
.158	.0847	.1798	.1583	.1492	.1313	.1645	.1448
.160	.0857	.1820	.1602	.1510	.1329	.1665	.1465
.162	.0868	.1842	.1621	.1528	.1345	.1685	.1483
.164	.0878	.1864	.1640	.1546	.1361	.1705	.1501
.166	.0888	.1886	.1660	.1565	.1377	.1726	.1519
.168	.0899	.1908	.1679	.1583	.1393	.1746	.1537
.170	.0909	.1930	.1698	.1601	.1409	.1766	.1554
.172	.0920	.1952	.1717	.1619	.1425	.1786	.1572
.174	.0930	.1974	.1736	.1637	.1441	.1806	.1590
.176	.0940	.1996	.1756	.1656	.1457	.1826	.1607
.178	.0951	.2018	.1775	.1674	.1473	.1846	.1625
.180	.0961	.2039	.1794	.1692	.1489	.1866	.1642
.182	.0971	.2061	.1813	.1710	.1505	.1886	.1660
.184	.0982	.2082	.1832	.1728	.1521	.1906	.1678
.186	.0992	.2104	.1851	.1747	.1537	.1926	.1695
.188	.1003	.2126	.1870	.1765	.1553	.1946	.1712
.190	.1013	.2147	.1889	.1783	.1569	.1965	.1729
.192	.1023	.2168	.1908	.1801	.1585	.1985	.1747
.194	.1034	.2190	.1927	.1819	.1601	.2005	.1764
.196	.1044	.2212	.1946	.1838	.1617	.2025	.1782
.198	.1054	.2233	.1965	.1856	.1633	.2045	.1800
.200	.1065	.2255	.1984	.1874	.1649	.2065	.1817
.202	.1075	.2276	.2003	.1892	.1665	.2085	.1835
.204	.1085	.2298	.2022	.1910	.1681	.2105	.1853
.206	.1096	.2320	.2041	.1929	.1697	.2125	.1869
.208	.1106	.2341	.2060	.1947	.1713	.2144	.1887

Kröber's table.—Concluded.

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(Expressed in grams.)

FURFURAL PHLOROGUCIDE	FURFURAL	ARABINOSE	ARABAN	XYLOSE	XYLAN	PENTOSE	PENTOSAN
0.210	0.1116	0.2363	0.2079	0.1965	0.1729	0.2164	0.1904
.212	.1127	.2384	.2098	.1984	.1745	.2184	.1922
.214	.1137	.2406	.2117	.2002	.1761	.2204	.1940
.216	.1147	.2428	.2136	.2020	.1778	.2224	.1957
.218	.1158	.2449	.2155	.2038	.1794	.2244	.1974
.220	.1168	.2471	.2174	.2057	.1810	.2264	.1992
.222	.1178	.2492	.2193	.2075	.1826	.2284	.2010
.224	.1189	.2514	.2212	.2093	.1842	.2304	.2028
.226	.1199	.2536	.2232	.2111	.1858	.2324	.2046
.228	.1209	.2557	.2251	.2130	.1874	.2344	.2063
.230	.1220	.2579	.2270	.2148	.1890	.2364	.2081
.232	.1230	.2600	.2289	.2166	.1906	.2383	.2097
.234	.1240	.2622	.2308	.2184	.1922	.2403	.2115
.236	.1251	.2644	.2327	.2202	.1938	.2423	.2132
.238	.1261	.2665	.2346	.2220	.1954	.2443	.2150
.240	.1271	.2687	.2365	.2239	.1970	.2463	.2168
.242	.1281	.2708	.2384	.2257	.1986	.2483	.2185
.244	.1292	.2730	.2403	.2275	.2002	.2503	.2203
.246	.1302	.2752	.2422	.2293	.2018	.2523	.2220
.248	.1312	.2773	.2441	.2311	.2034	.2543	.2238
.250	.1323	.2795	.2460	.2330	.2050	.2563	.2256
.252	.1333	.2816	.2479	.2348	.2066	.2582	.2272
.254	.1343	.2838	.2498	.2366	.2082	.2602	.2290
.256	.1354	.2860	.2517	.2384	.2098	.2622	.2307
.258	.1364	.2881	.2536	.2402	.2114	.2642	.2325
.260	.1374	.2903	.2555	.2420	.2130	.2662	.2342
.262	.1385	.2924	.2574	.2438	.2146	.2681	.2359
.264	.1395	.2946	.2593	.2456	.2162	.2701	.2377
.266	.1405	.2968	.2612	.2474	.2178	.2721	.2394
.268	.1416	.2989	.2631	.2492	.2194	.2741	.2412
.270	.1426	.3011	.2650	.2511	.2210	.2761	.2429
.272	.1436	.3032	.2669	.2529	.2226	.2781	.2447
.274	.1447	.3054	.2688	.2547	.2242	.2801	.2465
.276	.1457	.3076	.2707	.2565	.2258	.2821	.2482
.278	.1467	.3097	.2726	.2583	.2274	.2840	.2499
.280	.1478	.3119	.2745	.2602	.2290	.2861	.2517
.282	.1488	.3140	.2764	.2620	.2306	.2880	.2534
.284	.1498	.3162	.2783	.2638	.2322	.2900	.2552
.286	.1509	.3184	.2802	.2656	.2338	.2920	.2570
.288	.1519	.3205	.2821	.2674	.2354	.2940	.2587
.290	.1529	.3227	.2840	.2693	.2370	.2960	.2605
.292	.1540	.3248	.2859	.2711	.2386	.2980	.2622
.294	.1550	.3270	.2878	.2729	.2402	.3000	.2640
.296	.1560	.3292	.2897	.2747	.2418	.3020	.2658
.298	.1571	.3313	.2916	.2765	.2434	.3040	.2675
.300	.1581	.3335	.2935	.2784	.2450	.3060	.2693

19 Percentages by volume at 60°F of ethyl alcohol corresponding to apparent specific gravities at various temperatures.<sup>1</sup>

APPARENT SPECIFIC GRAVITY	15.56 15.56	20.20	22.22	24.24	25.25	26.26	28.28	30.30	32.32	34.34	35.35	36.36
1.0000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
.9999	.07	.07	.07	.07	.07	.07	.07	.07	.07	.07	.07	.07
.98	.13	.13	.13	.13	.13	.13	.13	.13	.13	.13	.13	.13
.97	.20	.20	.20	.20	.20	.20	.20	.20	.20	.20	.20	.20
.96	.27	.26	.26	.26	.26	.26	.26	.26	.26	.26	.26	.26
.95	.33	.33	.33	.33	.33	.33	.33	.33	.33	.33	.33	.33
.94	.40	.40	.40	.40	.40	.40	.40	.40	.40	.40	.40	.40
.93	.47	.46	.46	.46	.46	.46	.46	.46	.46	.46	.46	.46
.92	.53	.53	.53	.53	.53	.53	.53	.53	.53	.53	.53	.53
.91	.60	.60	.60	.60	.60	.60	.60	.60	.60	.60	.60	.60
.90	.67	.66	.66	.66	.66	.66	.66	.66	.66	.66	.66	.66
.89	.73	.73	.73	.73	.73	.73	.73	.73	.73	.73	.73	.73
.88	.80	.80	.80	.80	.80	.80	.79	.79	.79	.79	.79	.79
.87	.87	.87	.87	.87	.87	.87	.86	.86	.86	.86	.86	.86
.86	.93	.93	.93	.93	.93	.93	.93	.93	.93	.93	.93	.93
.85	1.00	1.00	1.00	1.00	1.00	1.00	.99	.99	.99	.99	.99	.99
.84	.07	.07	.07	.07	.07	.07	1.06	1.06	1.06	1.06	1.06	1.06
.83	.14	.14	.14	.13	.13	.13	.13	.13	.13	.13	.13	.13
.82	.20	.20	.20	.20	.20	.20	.20	.19	.19	.19	.19	.19
.81	.27	.27	.27	.27	.27	.27	.26	.26	.26	.26	.26	.26
.80	.34	.34	.34	.34	.34	.33	.33	.32	.32	.32	.32	.32
.79	.41	.41	.41	.40	.40	.40	.39	.39	.39	.39	.39	.39
.78	.48	.48	.48	.47	.47	.47	.46	.46	.46	.46	.46	.46
.77	.54	.54	.54	.54	.53	.53	.53	.53	.53	.53	.52	.52
.76	.61	.61	.61	.60	.60	.60	.59	.59	.59	.59	.59	.59
.75	.68	.68	.68	.67	.67	.67	.66	.66	.66	.66	.66	.66
.74	.75	.75	.75	.74	.74	.73	.73	.73	.73	.72	.72	.72
.73	.82	.81	.81	.81	.81	.80	.80	.80	.80	.79	.79	.79
.72	.88	.88	.88	.87	.87	.86	.86	.86	.86	.85	.85	.85
.71	.95	.95	.95	.94	.94	.94	.93	.93	.93	.92	.92	.92
.70	2.02	2.02	2.02	2.01	2.01	2.00	2.00	2.00	.99	.99	.99	.99
.69	.09	.09	.09	.08	.08	.08	.07	.07	.06	2.05	2.05	2.05
.68	.16	.15	.15	.14	.14	.14	.14	.14	.13	.12	.12	.12
.67	.23	.22	.22	.21	.21	.21	.20	.20	.20	.19	.19	.19
.66	.30	.29	.29	.28	.28	.28	.27	.27	.27	.26	.26	.26
.65	.37	.36	.36	.35	.35	.35	.34	.34	.33	.32	.32	.32
.64	.43	.43	.43	.42	.42	.42	.41	.41	.40	.39	.39	.39
.63	.50	.50	.50	.49	.49	.49	.48	.48	.47	.46	.46	.46
.62	.57	.57	.57	.56	.56	.56	.55	.54	.54	.53	.53	.53
.61	.64	.64	.64	.63	.63	.63	.62	.61	.60	.59	.59	.59
.60	.71	.70	.70	.70	.70	.70	.69	.68	.67	.67	.66	.66
.59	.78	.77	.77	.77	.77	.77	.76	.75	.74	.74	.73	.73
.58	.85	.84	.84	.83	.83	.83	.82	.82	.81	.81	.80	.80
.57	.92	.91	.91	.90	.90	.90	.89	.88	.87	.87	.86	.86
.56	.99	.98	.98	.97	.97	.97	.96	.95	.94	.94	.93	.93
.55	3.06	3.05	3.05	3.04	3.04	3.04	3.03	3.02	3.01	3.01	3.00	3.00
.54	.13	.12	.12	.11	.11	.11	.10	.09	.08	.08	.07	.07
.53	.20	.19	.19	.18	.18	.18	.17	.16	.15	.15	.14	.14
.52	.27	.26	.26	.25	.25	.25	.24	.23	.22	.22	.21	.21
.51	.34	.33	.33	.32	.32	.32	.31	.30	.29	.28	.27	.27
.50	.41	.40	.40	.39	.39	.39	.38	.37	.36	.35	.34	.34
.49	.49	.47	.47	.46	.46	.46	.45	.44	.43	.42	.41	.41
.48	.56	.54	.54	.53	.53	.53	.52	.51	.50	.49	.48	.48
.47	.63	.61	.61	.60	.60	.60	.59	.58	.57	.56	.55	.55
.46	.70	.68	.68	.67	.67	.67	.66	.65	.64	.63	.62	.62
.45	.77	.75	.75	.74	.74	.74	.73	.72	.70	.69	.68	.68
.44	.84	.83	.82	.81	.81	.81	.79	.78	.77	.76	.75	.75
.43	.91	.90	.89	.88	.88	.88	.86	.85	.84	.83	.82	.82
.42	.99	.97	.96	.95	.95	.95	.93	.92	.91	.90	.89	.89
.41	4.06	4.04	4.03	4.02	4.02	4.02	4.00	.99	.98	.97	.96	.96
.40	.13	.11	.10	.10	.09	.09	.07	4.06	4.05	4.04	4.03	4.03
.39	.20	.18	.17	.17	.16	.16	.14	.13	.12	.11	.10	.10
.38	.28	.26	.25	.25	.24	.23	.21	.20	.19	.18	.17	.17
.37	.35	.33	.32	.32	.31	.30	.28	.27	.26	.25	.24	.24
.36	.42	.40	.39	.39	.38	.37	.36	.35	.33	.32	.31	.30
.35	.50	.49	.47	.46	.45	.44	.43	.42	.40	.39	.38	.37
.34	.57	.55	.54	.53	.52	.51	.50	.49	.47	.46	.45	.44
.33	.64	.62	.61	.60	.59	.58	.57	.56	.54	.53	.52	.51
.32	.71	.69	.68	.67	.66	.65	.64	.63	.61	.60	.59	.58
.31	.79	.77	.76	.75	.74	.73	.72	.70	.68	.67	.66	.65
.30	.86	.84	.83	.82	.81	.80	.79	.77	.75	.74	.73	.72

<sup>1</sup> Compiled at the National Bureau of Standards. The table is based on data published in the Bulletin of the Bureau of Standards, Vol. 9, No. 3 (Reprint No. 197).

REFERENCE TABLES

XLII

Percentages by volume at 60°F of ethyl alcohol corresponding to apparent specific gravities at various temperatures.—Continued.

APPARENT SPECIFIC GRAVITY	15.56	20/20	22/22	24/24	25/25	26/26	28/28	30/30	32/32	34/34	35/35	36/36
	15.56											
0.9950	4.86	4.84	.83	.82	4.81	.80	.79	4.77	.75	.74	4.73	.72
29	.93	.91	.90	.89	.88	.87	.86	.84	.82	.81	.80	.79
28	5.01	.98	.97	.96	.95	.94	.93	.91	.89	.88	.87	.86
27	.08	5.06	5.04	5.03	5.02	5.01	5.00	.98	.96	.95	.94	.93
26	.16	.13	.12	.11	.10	.09	.07	5.05	5.03	5.02	5.01	5.00
25	.23	.21	.19	.18	.17	.16	.14	.12	.10	.09	.08	.07
24	.31	.28	.26	.25	.24	.23	.21	.20	.18	.16	.15	.14
23	.39	.36	.34	.33	.32	.31	.29	.27	.25	.23	.22	.21
22	.46	.43	.41	.40	.39	.38	.36	.34	.32	.30	.29	.28
21	.54	.51	.49	.48	.47	.46	.44	.42	.40	.38	.37	.36
20	.61	.58	.56	.55	.54	.53	.51	.49	.47	.45	.44	.43
19	.69	.66	.64	.62	.61	.60	.58	.56	.54	.52	.51	.50
18	.77	.73	.71	.70	.69	.68	.66	.64	.62	.59	.58	.57
17	.84	.81	.79	.77	.76	.75	.73	.71	.69	.66	.65	.64
16	.92	.88	.86	.85	.84	.83	.80	.78	.76	.74	.73	.72
15	.99	.96	.94	.92	.91	.90	.87	.85	.83	.81	.80	.79
14	6.07	6.03	6.01	6.00	.99	.98	.95	.93	.91	.88	.87	.86
13	.15	.11	.09	.07	6.06	6.05	6.02	6.00	.98	.95	.94	.93
12	.23	.18	.16	.15	.14	.13	.10	.08	6.05	6.02	6.04	6.00
11	.30	.26	.24	.22	.21	.20	.17	.15	.12	.10	.09	.08
10	.38	.34	.32	.30	.29	.28	.25	.23	.20	.17	.16	.15
09	.46	.41	.39	.37	.36	.35	.32	.30	.28	.25	.24	.23
08	.54	.49	.47	.45	.44	.43	.40	.38	.35	.32	.31	.30
07	.62	.57	.55	.53	.52	.51	.48	.45	.42	.39	.38	.37
06	.70	.65	.63	.60	.59	.58	.55	.53	.50	.47	.46	.45
05	.77	.73	.71	.68	.67	.66	.63	.60	.57	.54	.53	.52
04	.85	.80	.78	.75	.74	.73	.70	.68	.65	.62	.60	.59
03	.93	.88	.86	.83	.82	.81	.78	.75	.72	.69	.68	.67
02	7.01	.96	.93	.90	.89	.88	.85	.83	.80	.77	.75	.74
01	.09	7.04	7.01	.98	.97	.95	.92	.90	.87	.84	.82	.81
00	.17	.12	.09	7.06	7.05	7.03	7.00	.98	.94	.91	.90	.88
0.9899	.25	.19	.16	.13	.12	.10	.07	7.05	7.01	.98	.97	.95
98	.33	.27	.24	.21	.20	.18	.15	.13	.09	7.06	7.04	7.02
97	.41	.35	.32	.29	.28	.26	.23	.21	.17	.14	.12	.10
96	.50	.43	.40	.37	.36	.34	.31	.28	.24	.21	.19	.17
95	.58	.51	.48	.45	.44	.42	.39	.39	.32	.29	.27	.25
94	.66	.59	.56	.53	.52	.50	.47	.44	.40	.36	.34	.32
93	.74	.67	.64	.60	.59	.57	.54	.51	.47	.44	.42	.40
92	.82	.75	.72	.68	.67	.65	.62	.59	.55	.51	.49	.47
91	.90	.82	.79	.76	.75	.73	.70	.68	.62	.59	.57	.55
90	.98	.90	.87	.84	.83	.81	.78	.74	.70	.65	.64	.62
89	8.07	.98	.95	.92	.91	.89	.86	.82	.78	.74	.72	.70
88	.15	8.06	8.03	8.00	.98	.96	.93	.89	.85	.81	.79	.77
87	.23	.15	.11	.08	8.06	8.04	8.01	.97	.93	.89	.87	.85
86	.32	.23	.19	.16	.14	.12	.09	8.05	8.01	.96	.94	.92
85	.40	.31	.27	.24	.22	.20	.16	.12	.08	8.04	8.02	8.00
84	.48	.39	.35	.32	.30	.28	.24	.20	.16	.11	.09	.07
83	.57	.47	.43	.40	.38	.36	.32	.27	.23	.19	.17	.15
82	.65	.55	.51	.48	.46	.44	.40	.35	.31	.26	.24	.22
81	.73	.63	.59	.56	.54	.52	.48	.43	.39	.34	.32	.30
80	.82	.71	.67	.63	.61	.59	.55	.50	.46	.41	.39	.37
79	.90	.79	.75	.71	.69	.67	.63	.58	.54	.49	.47	.45
78	.98	.88	.84	.79	.77	.75	.71	.66	.61	.56	.54	.52
77	9.07	.96	.92	.87	.85	.83	.78	.73	.69	.64	.62	.60
76	.15	9.04	9.00	.85	.83	.81	.76	.71	.67	.62	.60	.58
75	.24	.13	.08	9.03	9.01	.99	.94	.89	.84	.79	.77	.75
74	.32	.21	.16	.11	.09	9.07	9.02	.96	.91	.86	.84	.82
73	.40	.29	.24	.19	.17	.15	.10	9.04	.99	.94	.92	.90
72	.49	.38	.33	.27	.25	.23	.18	.12	9.07	9.02	.96	.92
71	.57	.46	.41	.35	.33	.31	.26	.20	.15	.10	9.07	9.05
70	.66	.54	.49	.43	.41	.38	.33	.27	.22	.17	.14	.12
69	.74	.62	.57	.51	.49	.46	.41	.35	.30	.25	.22	.19
68	.82	.70	.65	.59	.57	.54	.49	.43	.37	.32	.29	.26
67	.91	.79	.74	.68	.65	.62	.57	.51	.45	.40	.37	.34
66	.99	.87	.82	.76	.73	.70	.65	.59	.53	.47	.44	.41
65	10.08	.95	.90	.84	.81	.78	.72	.66	.60	.54	.51	.48
64	.16	10.03	.98	.92	.89	.86	.80	.74	.68	.62	.59	.56
63	.25	.11	10.06	10.00	.97	.94	.88	.82	.76	.69	.66	.63
62	.33	.20	.14	.08	10.05	10.02	.96	.90	.84	.77	.74	.71
61	.42	.28	.22	.16	.13	.10	10.04	.98	.91	.84	.81	.78
60	.50	.36	.30	.24	.21	.18	.11	10.05	.99	.92	.89	.86



19 Percentages by volume at 60°F of ethyl alcohol corresponding to apparent specific gravities at various temperatures.—Continued.

APPARENT SPECIFIC GRAVITY	15.56 15.50	20/20	22/22	24/24	25/25	26/26	28/28	30/30	32/32	34/34	35/35	36/36
0.9860	10.50	10.36	.30	.24	10.21	.18	.11	10.05	.99	.92	0.89	.86
50	.59	.44	.38	.32	.29	.26	.19	.13	10.06	.99	.96	.93
58	.68	.53	.47	.40	.37	.34	.27	.21	.14	10.07	10.04	10.00
57	.76	.61	.55	.48	.44	.41	.34	.28	.21	.14	.11	.07
56	.85	.69	.63	.56	.52	.49	.42	.36	.29	.22	.19	.15
55	.93	.78	.71	.64	.60	.57	.50	.44	.37	.30	.26	.23
54	11.02	.86	.79	.72	.68	.65	.58	.52	.45	.38	.34	.31
53	.11	.94	.87	.80	.76	.73	.66	.59	.52	.45	.41	.38
52	.19	11.03	.96	.88	.84	.81	.74	.67	.60	.53	.49	.45
51	.28	.11	11.04	.96	.92	.89	.82	.75	.67	.60	.56	.52
50	.37	.19	.12	11.04	11.00	.96	.89	.82	.74	.67	.63	.59
49	.46	.28	.20	.12	.08	11.04	.97	.90	.82	.75	.71	.67
48	.54	.36	.28	.20	.16	.12	11.05	.98	.90	.82	.78	.74
47	.63	.45	.36	.28	.24	.20	.13	11.05	.97	.90	.86	.82
46	.72	.53	.45	.37	.33	.29	.21	.13	11.05	.97	.93	.89
45	.81	.61	.53	.45	.41	.37	.29	.21	.13	11.05	11.01	.97
44	.89	.70	.62	.53	.49	.45	.37	.29	.21	.12	.08	11.04
43	.98	.78	.70	.61	.57	.53	.44	.36	.28	.20	.16	.12
42	12.07	.87	.78	.69	.65	.61	.52	.44	.36	.27	.23	.19
41	.16	.95	.86	.78	.73	.69	.60	.52	.44	.35	.31	.27
40	.25	12.04	.95	.86	.81	.77	.68	.60	.51	.42	.38	.34
39	.34	12.03	.94	.89	.85	.80	.76	.67	.58	.50	.46	.42
38	.43	.21	.12	12.03	.98	.93	.84	.75	.66	.57	.53	.49
37	.52	.29	.20	.11	12.06	12.01	.92	.83	.74	.65	.61	.57
36	.61	.38	.28	.19	.14	.09	12.00	.91	.82	.73	.68	.64
35	.70	.47	.37	.27	.22	.17	.07	.98	.89	.80	.76	.72
34	.79	.55	.45	.35	.30	.25	.15	12.06	.97	.88	.83	.79
33	.88	.64	.54	.44	.39	.34	.24	.14	12.05	.96	.91	.86
32	.97	.73	.63	.52	.47	.42	.32	.22	.12	12.03	.98	.93
31	13.06	.81	.71	.60	.55	.50	.40	.30	.20	.11	12.06	12.01
30	.16	.90	.79	.68	.63	.58	.48	.38	.28	.19	.14	.09
29	.25	.99	.88	.77	.71	.66	.56	.46	.36	.26	.21	.16
28	.34	13.07	.96	.85	.80	.74	.64	.54	.44	.34	.29	.24
27	.43	.16	13.05	.93	.88	.82	.72	.62	.52	.42	.37	.32
26	.52	.25	.13	13.01	.96	.90	.80	.70	.59	.49	.44	.39
25	.61	.34	.22	.10	13.04	.90	.88	.78	.67	.57	.52	.47
24	.71	.43	.31	.19	.13	13.08	.97	.86	.75	.65	.60	.55
23	.80	.51	.39	.27	.21	.16	13.05	.94	.83	.72	.67	.62
22	.89	.60	.47	.35	.29	.23	.13	13.02	.91	.80	.75	.70
21	.98	.68	.56	.44	.38	.33	.22	.10	.99	.88	.82	.77
20	14.08	.77	.64	.52	.46	.40	.29	.18	13.06	.95	.90	.85
19	.17	.86	.73	.61	.55	.49	.37	.26	.15	13.04	.98	.93
18	.26	.95	.82	.69	.63	.57	.45	.34	.22	.11	13.05	13.00
17	.36	14.04	.91	.78	.72	.66	.54	.42	.30	.19	.13	.08
16	.45	.13	14.00	.87	.80	.74	.62	.50	.38	.27	.21	.16
15	.55	.22	.08	.65	.88	.82	.70	.58	.46	.34	.28	.23
14	.64	.30	.17	14.04	.97	.91	.78	.66	.54	.42	.36	.30
13	.74	.39	.25	.12	14.05	.99	.86	.74	.62	.50	.44	.38
12	.83	.48	.34	.20	.13	14.07	.94	.82	.70	.58	.52	.46
11	.92	.57	.43	.29	.22	.16	14.03	.90	.77	.65	.59	.53
10	15.02	.66	.51	.37	.30	.24	.11	.98	.85	.73	.67	.61
09	.11	.75	.60	.46	.39	.32	.19	14.06	.93	.81	.75	.69
08	.21	.84	.79	.64	.47	.40	.27	.14	14.01	.88	.82	.76
07	.30	.93	.87	.62	.55	.48	.35	.22	.09	.90	.84	.78
06	.40	15.02	.76	.71	.64	.57	.43	.30	.17	14.04	.98	.92
05	.49	.11	.95	.79	.72	.65	.51	.38	.25	.12	14.05	.99
04	.58	.20	15.04	.88	.81	.74	.60	.46	.33	.20	.13	14.07
03	.67	.28	.12	.96	.89	.82	.68	.54	.41	.28	.21	.15
02	.77	.37	.21	15.05	.97	.90	.76	.62	.49	.36	.29	.23
01	.87	.46	.30	.14	15.06	.99	.84	.70	.56	.43	.36	.30
00	.96	.55	.39	.23	.15	15.07	.92	.78	.64	.51	.44	.38
0.9799	16.06	.64	.48	.32	.24	.16	15.01	.86	.72	.59	.52	.46
98	.15	.73	.46	.40	.32	.24	.09	.94	.80	.67	.60	.54
97	.25	.82	.55	.49	.41	.33	.17	15.02	.88	.74	.67	.61
96	.35	.91	.64	.57	.49	.41	.26	.11	.96	.83	.75	.68
95	.44	16.00	.83	.66	.58	.50	.34	.19	15.04	.90	.83	.76
94	.54	.10	.92	.75	.66	.59	.43	.27	.12	.98	.91	.84
93	.63	.19	16.01	.84	.75	.67	.51	.35	.20	15.05	.98	.91
92	.73	.28	.10	.93	.84	.76	.59	.43	.28	.13	15.06	.99
91	.83	.37	.19	10.01	.92	.84	.67	.51	.36	.21	.14	15.07
90	.92	.46	.27	.09	16.00	.92	.75	.59	.44	.29	.22	.15

Percentages by volume at 60°F of ethyl alcohol corresponding to apparent 19  
specific gravities at various temperatures.—Continued.

APPARENT SPECIFIC GRAVITY	15.56 15.56	20/20	22/22	24/24	25/25	26/26	28/28	30/30	32/32	34/34	35/35	36/36
0.9790	18.92	16.46	16.27	16.09	16.00	15.92	15.75	15.59	15.44	15.29	15.22	15.15
89	17.02	.55	.26	.18	.09	16.01	.84	.67	.52	.37	.30	.23
88	.12	.64	.45	.27	.18	.10	.93	.76	.61	.45	.38	.31
87	.22	.73	.54	.36	.27	.18	16.01	.84	.68	.52	.45	.38
86	.32	.83	.63	.44	.35	.26	.09	.92	.76	.60	.53	.46
85	.42	.92	.72	.53	.44	.35	.17	16.06	.84	.68	.61	.53
84	.51	17.01	.81	.62	.53	.44	.26	.08	.92	.76	.69	.61
83	.61	.10	.90	.70	.61	.52	.34	.17	.10	.84	.77	.69
82	.71	.20	.99	.79	.70	.61	.43	.25	16.08	.92	.84	.76
81	.81	.29	17.08	.88	.78	.69	.51	.33	.16	16.00	.92	.84
80	.91	.38	.17	.97	.87	.78	.59	.41	.24	.08	16.00	.92
79	18.01	.47	.26	17.06	.96	.87	.68	.50	.33	.16	.08	16.00
78	.11	.57	.35	.14	17.04	.95	.76	.58	.41	.24	.16	.08
77	.21	.66	.44	.23	.13	17.04	.85	.66	.49	.32	.24	.16
76	.31	.75	.53	.32	.22	.12	.93	.74	.57	.40	.32	.24
75	.41	.84	.62	.40	.30	.20	17.01	.83	.65	.48	.40	.32
74	.51	.94	.72	.50	.39	.29	.10	.91	.73	.56	.48	.40
73	.61	18.03	.81	.59	.48	.38	.18	.99	.81	.64	.56	.48
72	.71	.12	.90	.68	.57	.47	.27	17.07	.89	.72	.63	.55
71	.81	.22	.99	.76	.65	.55	.35	.16	.97	.80	.71	.63
70	.91	.31	18.08	.85	.74	.63	.43	.24	17.05	.88	.79	.71
69	19.01	.40	.16	.94	.83	.72	.52	.32	.14	.96	.87	.79
68	.11	.50	.25	18.02	.91	.80	.60	.40	.22	17.04	.95	.86
67	.21	.59	.34	.11	18.00	.89	.69	.49	.30	.12	17.03	.94
66	.32	.69	.44	.20	.09	.98	.78	.57	.38	.20	.11	17.02
65	.42	.78	.53	.29	.18	18.07	.86	.65	.46	.28	.19	.10
64	.52	.88	.63	.38	.27	.16	.95	.74	.55	.36	.27	.17
63	.62	.97	.71	.47	.35	.24	18.03	.82	.62	.43	.35	.25
62	.72	19.07	.81	.56	.44	.33	.11	.90	.70	.51	.43	.33
61	.83	.16	.80	.65	.53	.42	.20	.98	.78	.59	.50	.41
60	.93	.26	.99	.74	.62	.50	.28	18.07	.87	.67	.58	.49
59	20.03	.35	19.08	.83	.71	.60	.37	.15	.95	.75	.66	.56
58	.13	.45	.18	.92	.80	.69	.46	.23	18.03	.83	.74	.64
57	.23	.54	.27	19.01	.88	.77	.54	.32	.11	.91	.82	.72
56	.33	.64	.36	.10	.97	.86	.62	.40	.19	.99	.90	.80
55	.43	.73	.45	.19	19.06	.94	.70	.48	.27	18.07	.98	.88
54	.53	.83	.55	.28	.15	19.03	.79	.57	.36	.15	18.06	.96
53	.63	.92	.64	.37	.24	.12	.88	.65	.44	.23	.13	18.04
52	.73	20.02	.73	.46	.33	.21	.96	.73	.52	.31	.21	.12
51	.83	.11	.82	.55	.42	.30	19.05	.82	.60	.39	.29	.19
50	.93	.20	.91	.64	.50	.38	.13	.90	.68	.47	.37	.27
49	21.03	.30	20.01	.73	.59	.47	.22	.98	.76	.55	.45	.35
48	.13	.39	.10	.82	.68	.56	.31	19.07	.85	.64	.53	.43
47	.23	.48	.19	.91	.77	.65	.39	.15	.93	.72	.61	.51
46	.33	.58	.28	20.00	.86	.74	.48	.24	19.01	.80	.69	.59
45	.43	.67	.37	.09	.95	.82	.56	.32	.09	.88	.77	.67
44	.52	.76	.46	.17	20.03	.90	.64	.40	.17	.96	.85	.75
43	.62	.86	.55	.26	.12	.99	.73	.49	.26	19.04	.93	.83
42	.72	.95	.64	.35	.21	20.08	.82	.57	.34	.12	19.01	.91
41	.82	21.04	.73	.44	.30	.17	.91	.66	.42	.20	.09	.98
40	.92	.14	.82	.53	.38	.25	.99	.74	.50	.28	.17	19.06
39	22.02	.23	.91	.62	.47	.34	20.07	.82	.58	.35	.24	.23
38	.12	.32	21.00	.71	.56	.43	.16	.90	.66	.43	.32	.31
37	.22	.41	.09	.79	.64	.51	.24	.08	.74	.51	.40	.29
36	.31	.50	.18	.88	.73	.59	.32	.20	.82	.59	.48	.37
35	.41	.60	.27	.97	.82	.68	.41	.15	.90	.67	.56	.45
34	.51	.69	.36	21.05	.90	.77	.50	.24	.99	.75	.64	.53
33	.61	.78	.45	.14	.99	.85	.58	.32	20.07	.83	.72	.61
32	.71	.87	.54	.23	21.08	.94	.66	.40	.15	.91	.80	.68
31	.80	.96	.63	.32	.16	21.02	.74	.48	.23	.99	.87	.76
30	.90	22.05	.72	.41	.25	.11	.83	.56	.31	20.07	.95	.84
29	23.00	.14	.81	.50	.34	.20	.91	.64	.39	.15	20.03	.92
28	.10	.24	.90	.58	.42	.28	.99	.72	.47	.23	.11	20.00
27	.19	.33	.99	.67	.51	.38	21.07	.80	.55	.31	.19	.08
26	.28	.42	22.08	.76	.59	.45	.16	.89	.63	.39	.27	.16
25	.38	.51	.17	.84	.68	.53	.24	.07	.71	.46	.34	.23
24	.48	.60	.26	.93	.77	.62	.33	21.05	.79	.54	.42	.30
23	.58	.69	.34	22.01	.86	.70	.41	.13	.87	.62	.50	.38
22	.67	.78	.43	.10	.94	.78	.49	.21	.95	.70	.58	.46
21	.77	.87	.52	.19	22.03	.87	.58	.30	21.03	.78	.66	.54
20	.87	.96	.61	.27	.11	.96	.66	.38	.11	.86	.73	.61

19 Percentages by volume at 60°F of ethyl alcohol corresponding to apparent specific gravities at various temperatures.—Continued.

APPARENT SPECIFIC GRAVITY	15.56	20.20	22.22	24.24	25.25	26.26	28.28	30.30	32.32	34.34	35.35	36.36
	15.56											
0.9720	23.87	22.96	.61	.27	22.11	.96	.66	21.38	.11	.86	20.73	.61
19	.96	23.06	.70	.36	.19	22.04	.74	.46	.19	.94	.81	.69
18	24.06	.15	.79	.45	.28	.12	.82	.54	.27	21.02	.89	.77
17	.15	.24	.88	.54	.36	.21	.91	.62	.35	.10	.97	.85
16	.25	.33	.96	.62	.45	.30	.99	.70	.43	.17	21.05	.92
15	.34	.42	23.05	.70	.53	.38	22.08	.79	.51	.24	.12	.99
14	.43	.51	.14	.79	.62	.46	.16	.87	.59	.33	.20	21.08
13	.53	.60	.22	.87	.70	.54	.24	.95	.67	.40	.27	.15
12	.62	.69	.31	.96	.79	.63	.32	22.03	.75	.88	.35	.22
11	.72	.78	.40	23.04	.87	.71	.40	.11	.83	.56	.43	.30
10	.81	.87	.49	.13	.96	.80	.49	.19	.91	.64	.50	.37
09	.91	.95	.57	.21	23.04	.88	.57	.27	.99	.72	.58	.45
08	25.00	24.04	.66	.30	.13	.97	.65	.35	22.07	.80	.66	.53
07	.09	.13	.74	.38	.21	23.05	.73	.43	.14	.87	.73	.60
06	.19	.22	.83	.47	.29	.13	.81	.51	.22	.95	.81	.68
05	.28	.31	.92	.56	.38	.22	.90	.59	.30	22.03	.89	.76
04	.38	.40	24.00	.64	.46	.30	.98	.67	.38	.10	.96	.83
03	.47	.49	.09	.73	.55	.38	23.06	.75	.46	.18	22.04	.91
02	.57	.58	.18	.81	.63	.46	.14	.83	.53	.25	.11	.98
01	.66	.66	.26	.89	.71	.54	.21	.90	.61	.33	.19	22.06
00	.75	.75	.35	.98	.80	.63	.30	.98	.69	.41	.27	.14
0.9699	.85	.81	.41	24.06	.88	.72	.38	23.06	.77	.48	.34	.21
98	.94	.93	.53	.15	.97	.80	.46	.14	.84	.55	.42	.28
97	26.04	25.01	.61	.23	24.05	.88	.54	.22	.92	.63	.49	.35
96	.13	.10	.69	.31	.13	.96	.62	.30	23.00	.71	.57	.43
95	.22	.19	.78	.40	.22	24.05	.70	.38	.08	.78	.64	.50
94	.31	.28	.86	.48	.30	.13	.78	.45	.15	.86	.72	.58
93	.41	.36	.95	.57	.38	.21	.86	.53	.23	.94	.80	.66
92	.50	.45	25.04	.65	.47	.29	.94	.61	.31	23.01	.87	.74
91	.59	.54	.13	.74	.55	.37	24.02	.69	.38	.08	.95	.81
90	.69	.62	.21	.92	.63	.45	.10	.77	.46	.16	23.02	.88
89	.78	.71	.29	.90	.72	.53	.18	.84	.53	.23	.10	.96
88	.87	.80	.38	.98	.80	.61	.26	.92	.61	.31	23.03	.93
87	.96	.89	.46	25.07	.88	.69	.34	24.00	.68	.38	.24	.10
86	27.03	.98	.55	.15	.97	.77	.42	.08	.79	.46	.32	.18
85	.15	26.06	.63	.23	23.05	.85	.50	.16	.84	.53	.39	.25
84	.24	.15	.72	.32	.13	.94	.58	.23	.92	.61	.47	.33
83	.33	.24	.80	.40	.21	25.02	.66	.31	.99	.68	.54	.40
82	.42	.33	.89	.48	.29	.10	.74	.39	24.06	.75	.61	.47
81	.51	.41	.97	.57	.37	.18	.81	.47	.14	.85	.69	.54
80	.60	.50	26.06	.65	.45	.26	.89	.54	.21	.90	.76	.61
79	.69	.59	.11	.73	.53	.34	.97	.62	.30	.98	.84	.69
78	.78	.67	.22	.81	.61	.42	25.05	.70	.37	24.06	.91	.77
77	.87	.76	.31	.89	.69	.50	.13	.78	.45	.14	.96	.84
76	.96	.84	.39	.97	.77	.58	.21	.85	.32	.21	24.06	.91
75	28.05	.93	.47	26.05	.85	.66	.29	.93	.60	.29	.13	.99
74	.14	27.01	.56	.14	.94	.74	.37	25.01	.68	.36	.21	24.06
73	.23	.10	.64	.22	26.02	.82	.45	.09	.75	.43	.28	.13
72	.32	.19	.73	.30	.10	.90	.53	.16	.83	.51	.36	.20
71	.41	.27	.81	.38	.18	.98	.60	.24	.90	.58	.43	.28
70	.50	.36	.89	.46	.26	26.06	.68	.32	.98	.66	.50	.35
69	.59	.44	.97	.54	.34	.14	.76	.40	25.06	.73	.58	.42
68	.68	.52	27.05	.63	.42	.22	.84	.47	.13	.81	.65	.50
67	.77	.61	.14	.71	.50	.30	.92	.55	.20	.88	.73	.57
66	.86	.69	.22	.79	.58	.38	.99	.63	.28	.95	.80	.64
65	.95	.77	.30	.87	.66	.46	26.07	.70	.36	25.03	.87	.72
64	29.04	.85	.39	.95	.74	.54	.15	.78	.44	.11	.95	.79
63	.12	.94	.47	27.03	.82	.62	.23	.86	.51	.18	25.02	.86
62	.21	26.02	.55	.11	.90	.70	.31	.94	.59	.25	.09	.93
61	.30	.11	.64	.19	.98	.77	.38	26.02	.66	.33	.17	25.01
60	.39	.19	.72	.27	27.06	.85	.46	.09	.74	.40	.24	.08
59	.47	.28	.81	.35	.13	.93	.54	.17	.82	.48	.31	.15
58	.56	.36	.89	.43	.21	27.01	.61	.24	.89	.56	.39	.23
57	.65	.44	.97	.51	.29	.09	.69	.32	.97	.63	.46	.30
56	.74	.53	27.05	.59	.37	.17	.77	.39	26.04	.70	.53	.47
55	.82	.61	.13	.67	.45	.25	.85	.47	.11	.77	.61	.45
54	.91	.69	.21	.75	.53	.33	.93	.55	.19	.85	.68	.52
53	30.00	.78	.29	.83	.61	.41	27.00	.62	.26	.92	.75	.59
52	.09	.86	.37	.91	.69	.49	.08	.70	.54	.09	.82	.66
51	.17	.94	.45	.99	.77	.66	.16	.78	.41	26.06	.90	.74
50	.26	29.03	.53	28.07	.85	.64	.23	.85	.49	.14	.97	.81

Percentages by volume at 60°F of ethyl alcohol corresponding to apparent specific gravities at various temperatures.—Continued.

APPARENT SPECIFIC GRAVITY	15.56	20/20	22/22	24/24	25/25	26/26	28/28	30/30	32/32	34/34	35/35	36/36
	15.56											
0.9650	30.26	29.03	.53	.07	27.85	.64	.23	26.85	.40	.14	25.97	.81
49	.34	.11	.61	.15	.39	.72	.31	.92	.56	.21	26.04	.89
48	.43	.19	.69	.23	28.01	.79	.38	27.00	.64	.29	.11	.96
47	.52	.27	.73	.31	.09	.87	.46	.07	.71	.36	.19	26.03
46	.60	.35	.85	.39	.16	.95	.53	.15	.78	.43	.26	.10
45	.69	.44	.93	.47	.24	28.03	.61	.22	.85	.51	.33	.17
44	.78	.52	29.02	.55	.32	.10	.69	.30	.93	.58	.40	.24
43	.86	.60	.10	.63	.40	.18	.76	.37	27.00	.65	.47	.31
42	.95	.68	.18	.71	.47	.26	.84	.44	.07	.72	.54	.38
41	31.03	.76	.26	.79	.55	.34	.91	.52	.14	.79	.61	.45
40	.11	.85	.34	.86	.63	.41	.99	.59	.22	.86	.69	.52
39	.20	.93	.42	.93	.71	.49	28.06	.67	.29	.93	.76	.59
38	.28	30.01	.50	29.01	.78	.56	.14	.74	.37	27.01	.83	.66
37	.36	.09	.58	.09	.86	.64	.21	.81	.44	.08	.90	.73
36	.44	.17	.66	.17	.94	.72	.29	.89	.51	.15	.97	.80
35	.52	.25	.74	.25	29.02	.80	.37	.96	.58	.22	27.04	.87
34	.61	.34	.82	.33	.09	.87	.44	28.04	.66	.29	.11	.94
33	.69	.42	.90	.41	.17	.95	.52	.11	.73	.36	.18	27.01
32	.77	.50	.98	.49	.25	29.03	.60	.19	.80	.43	.25	.08
31	.85	.58	30.06	.57	.33	.11	.67	.26	.87	.50	.32	.15
30	.93	.66	.13	.64	.40	.18	.74	.33	.95	.58	.39	.22
29	32.02	.74	.21	.72	.48	.26	.82	.41	28.02	.65	.46	.29
28	.09	.82	.29	.79	.56	.33	.89	.48	.10	.72	.54	.36
27	.17	.89	.36	.87	.64	.41	.97	.56	.17	.79	.61	.43
26	.25	.97	.44	.95	.71	.48	29.04	.63	.24	.86	.68	.50
25	.33	31.05	.52	30.03	.79	.55	.12	.70	.31	.93	.75	.57
24	.41	.13	.60	.10	.87	.64	.20	.78	.38	28.00	.82	.64
23	.49	.20	.67	.17	.95	.71	.27	.85	.45	.07	.89	.71
22	.57	.28	.75	.25	30.02	.79	.35	.93	.52	.14	.96	.78
21	.65	.36	.83	.33	.10	.86	.42	29.00	.59	.21	28.03	.85
20	.72	.44	.91	.41	.17	.94	.50	.07	.67	.29	.10	.92
19	.80	.52	.21	.25	30.01	.57	.14	.74	.36	.17	.99	.83
18	.88	.59	.28	.32	.09	.65	.22	.82	.43	.24	28.06	.91
17	.96	.67	.35	.40	.16	.72	.29	.89	.50	.31	.13	.93
16	33.04	.75	.42	.47	.24	.79	.36	.96	.57	.38	.20	.94
15	.12	.82	.54	.31	.80	.43	.51	29.03	.64	.45	.27	.95
14	.19	.90	.62	.62	.30	.94	.51	.10	.71	.52	.34	.96
13	.27	.98	.69	.46	30.01	.58	.17	.78	.59	.41	.28	.97
12	.35	32.05	.77	.53	.08	.65	.24	.85	.66	.48	.25	.98
11	.43	.13	.84	.61	.15	.72	.31	.92	.73	.55	.31	.99
10	.50	.21	.92	.68	.23	.80	.39	.99	.80	.62	.35	.99
09	.58	.28	.99	.75	.30	.87	.46	29.06	.87	.69	.37	.99
08	.66	.36	31.07	.83	.38	.94	.53	.13	.94	.76	.39	.99
07	.74	.43	.14	.90	.45	10.01	.60	.20	29.01	.83	.41	.99
06	.81	.51	.21	.98	.52	.09	.67	.27	.08	.90	.43	.99
05	.89	.58	.29	31.05	.59	.16	.74	.34	.15	.97	.45	.99
04	.97	.66	.36	.56	.13	.66	.23	.81	.41	.22	29.04	.99
03	34.05	.73	.43	.20	.73	.30	.88	.48	.29	.11	.99	.99
02	.12	.81	.51	.28	.80	.37	.95	.55	.36	.18	.99	.99
01	.20	.88	.58	.35	.88	.44	30.02	.62	.43	.25	.99	.99
00	.27	.96	.65	.42	.95	.51	.09	.69	.50	.31	.99	.99
0.9599	.35	33.03	.73	.50	.80	.58	.16	.76	.57	.38	.99	.99
98	.42	.10	.80	.57	.87	.65	.23	.83	.63	.45	.99	.99
97	.50	.18	.87	.65	.95	.72	.30	.90	.70	.51	.99	.99
96	.57	.25	.95	.73	32.02	.79	.37	.97	.77	.58	.99	.99
95	.65	.32	.98	.80	.09	.87	.44	30.04	.84	.65	.99	.99
94	.72	.40	.16	.87	.16	.94	.51	.11	.91	.72	.99	.99
93	.80	.47	.23	.94	.23	31.01	.58	.18	.98	.79	.99	.99
92	.87	.54	.23	.99	.23	.08	.65	.25	30.05	.86	.99	.99
91	.95	.62	.30	.99	.30	.15	.72	.32	.12	.93	.99	.99
90	35.02	.69	.37	.99	.37	.22	.79	.38	.18	.99	.99	.99
89	.09	.76	.44	.99	.44	.28	.46	.25	30.06	.99	.99	.99
88	.17	.84	.51	.99	.51	.35	.53	.32	.13	.99	.99	.99
87	.24	.91	.58	.99	.58	.42	.60	.39	.20	.99	.99	.99
86	.31	.98	.65	.99	.65	.49	.67	.46	.27	.99	.99	.99
85	.38	34.05	.73	.99	.73	.56	.74	.53	.33	.99	.99	.99
84	.46	.12	.80	.99	.80	.63	.81	.60	.40	.99	.99	.99
83	.53	.20	.87	.99	.87	.70	.88	.67	.47	.99	.99	.99
82	.60	.27	.94	.99	.94	.77	.95	.74	.54	.99	.99	.99
81	.67	.34	33.01	.99	.99	.84	.99	.81	.61	.99	.99	.99
80	.75	.41	.08	.99	.99	.91	.99	.88	.67	.99	.99	.99

19 Percentages by volume at 60°F of ethyl alcohol corresponding to apparent specific gravities at various temperatures.—Continued.

APPARENT SPECIFIC GRAVITY	15.56	20/20	25/25	30/30	35/35	APPARENT SPECIFIC GRAVITY	15.56	20/20	25/25	30/30	35/35
	15.56						15.56				
0.9580	35.75	34.41	33.08	31.91	30.86	0.9510	40.46	39.10	37.71	36.47	35.34
79	.32	.48	.15	.98	.93	99	.52	.16	.73	.53	.40
78	.89	.36	.22	32.05	31.00	08	.58	.23	.84	.59	.46
77	.96	.63	.29	.11	.07	07	.65	.29	.90	.65	.52
76	36.04	.70	.36	.18	.13	06	.71	.35	.96	.72	.58
75	.11	.77	.45	.25	.20	05	.77	.41	38.02	.78	.64
74	.18	.84	.50	.32	.26	04	.84	.48	.09	.84	.71
73	.25	.91	.57	.38	.33	03	.90	.54	.15	.90	.77
72	.32	.98	.64	.45	.39	02	.96	.60	.21	.96	.83
71	.39	35.05	.71	.52	.46	01	41.02	.67	.27	37.02	.89
70	.46	.12	.78	.58	.53	00	.09	.73	.33	.09	.95
69	.53	.19	.85	.65	.59	0.9499	.15	.79	.59	.15	36.01
68	.60	.26	.92	.72	.66	98	.21	.85	.46	.21	.07
67	.67	.33	.99	.79	.72	97	.27	.91	.52	.27	.13
66	.74	.40	34.03	.85	.79	96	.33	.98	.58	.33	.19
65	.81	.47	.12	.92	.86	95	40.04	.04	.64	.39	.25
64	.88	.54	.19	.99	.92	94	.46	.10	.70	.45	.31
63	.95	.61	.26	33.05	.99	93	.52	.16	.77	.51	.37
62	37.02	.68	.32	.12	32.05	92	.58	.22	.83	.57	.43
61	.09	.75	.39	.19	.12	91	.64	.29	.89	.63	.49
60	.16	.82	.46	.25	.18	90	.70	.35	.95	.70	.55
59	.22	.88	.53	.32	.25	89	.77	.41	39.01	.76	.61
58	.29	.95	.59	.39	.31	88	.83	.47	.07	.82	.67
57	.36	36.02	.66	.45	.37	87	.89	.53	.13	.88	.73
56	.43	.09	.78	.52	.44	86	.95	.59	.20	.94	.79
55	.50	.15	.80	.59	.50	85	42.01	.65	.26	38.00	.85
54	.56	.22	.86	.68	.57	84	.07	.71	.32	.06	.91
53	.63	.29	.93	.72	.63	83	.13	.78	.38	.12	.97
52	.70	.36	35.00	.79	.70	82	.19	.84	.44	.18	37.03
51	.77	.42	.07	.85	.76	81	.25	.90	.50	.24	.09
50	.84	.49	.13	.92	.83	80	.31	.96	.56	.30	.15
49	.90	.56	.20	.94	.89	79	.37	41.02	.62	.36	.21
48	.97	.63	.26	34.05	.95	78	.43	.08	.68	.42	.26
47	38.01	.69	.33	.12	33.02	77	.49	.14	.74	.48	.32
46	.11	.76	.39	.18	.08	76	.55	.20	.80	.54	.38
45	.17	.83	.46	.25	.15	75	.61	.26	.87	.60	.44
44	.24	.89	.53	.31	.21	74	.67	.32	.93	.66	.50
43	.31	.96	.59	.38	.27	73	.73	.38	.99	.72	.56
42	.37	37.03	.66	.44	.34	72	.80	.44	40.05	.78	.62
41	.44	.09	.72	.51	.40	71	.86	.50	.11	.81	.68
40	.51	.16	.79	.57	.46	70	.92	.56	.17	.90	.74
39	.57	.23	.86	.64	.53	69	.98	.62	.22	.96	.79
38	.64	.29	.92	.70	.59	68	43.04	.68	.28	39.02	.85
37	.71	.36	.99	.77	.66	67	.09	.74	.34	.08	.91
36	.77	.42	36.05	.85	.72	66	.15	.80	.40	.13	.97
35	.84	.49	.12	.90	.78	65	.21	.86	.46	.19	38.03
34	.91	.56	.18	.98	.85	64	.27	.92	.52	.25	.09
33	.97	.62	.25	35.04	.91	63	.33	.98	.58	.31	.15
32	39.01	.69	.31	.09	.97	62	.39	42.04	.64	.37	.20
31	.10	.75	.38	.15	34.01	61	.45	.09	.70	.43	.26
30	.17	.82	.44	.22	.10	60	.51	.15	.76	.49	.32
29	.23	.88	.51	.28	.16	59	.57	.21	.82	.54	.38
28	.30	.95	.57	.34	.22	58	.63	.27	.88	.60	.44
27	.36	38.01	.61	.41	.29	57	.69	.33	.93	.66	.49
26	.43	.67	.70	.47	.35	56	.75	.39	.99	.72	.55
25	.49	.14	.77	.55	.41	55	.80	.45	41.03	.78	.61
24	.56	.20	.83	.62	.47	54	.86	.51	.11	.84	.67
23	.62	.27	.90	.66	.53	53	.92	.57	.17	.89	.73
22	.69	.33	.96	.72	.60	52	.98	.63	.23	.95	.78
21	.75	.39	37.02	.78	.66	51	44.04	.69	.28	40.01	.81
20	.82	.46	.09	.85	.72	50	.10	.74	.31	.07	.99
19	.88	.52	.15	.91	.78	49	.16	.80	.40	.13	.96
18	.95	.59	.21	.97	.84	48	.21	.86	.46	.18	39.02
17	40.01	.65	.28	36.01	.91	47	.27	.92	.51	.21	.07
16	.08	.72	.34	.10	.97	46	.33	.98	.57	.30	.13
15	.14	.78	.40	.16	35.01	45	.39	43.04	.63	.35	.19
14	.20	.84	.46	.22	.10	44	.45	.09	.79	.41	.24
13	.27	.91	.52	.28	.16	43	.50	.15	.75	.47	.30
12	.33	.97	.59	.35	.22	42	.56	.21	.80	.53	.36
11	.39	39.04	.65	.41	.28	41	.62	.27	.85	.58	.41
10	.46	.10	.71	.47	.34	40	.68	.33	.92	.64	.47

REFERENCE TABLES

XLII

Percentages by volume at 60°F of ethyl alcohol corresponding to apparent specific gravities at various temperatures.—Continued.

APPARENT SPECIFIC GRAVITY	15.56	20/20	25/25	30/30	35/35	APPARENT SPECIFIC GRAVITY	15.56	20/20	25/25	30/30	35/35
	15.56						15.56				
0.9440	44.68	43.33	41.92	40.64	39.47	0.9370	48.53	47.20	45.81	44.52	43.33
39	.73	.39	.98	.70	.53	69	.58	.26	.85	.58	.38
38	.79	.44	42.03	.75	.59	68	.63	.31	.91	.63	.43
37	.85	.50	.09	.81	.64	67	.69	.36	.97	.68	.49
36	.91	.56	.15	.87	.70	66	.74	.42	46.02	.74	.54
35	.97	.62	.21	.93	.76	65	.79	.47	.07	.79	.59
34	45.02	.67	.26	.98	.81	64	.85	.52	.13	.84	.65
33	.08	.73	.32	41.04	.87	63	.90	.58	.18	.90	.70
32	.14	.78	.38	.10	.93	62	.95	.63	.23	.95	.75
31	.19	.85	.43	.15	.98	61	49.01	.68	.29	45.01	.81
30	.25	.90	.49	.21	40.04	60	.06	.73	.34	.06	.88
29	.31	.96	.55	.27	.09	59	.11	.79	.39	.11	.91
28	.36	44.02	.61	.32	.15	58	.16	.84	.45	.16	.97
27	.42	.07	.66	.38	.21	57	.21	.89	.50	.22	44.02
26	.47	.13	.72	.44	.26	56	.26	.94	.55	.27	.07
25	.53	.18	.78	.49	.32	55	.32	48.00	.61	.32	.13
24	.59	.24	.83	.55	.37	54	.37	.05	.66	.37	.18
23	.64	.30	.89	.60	.43	53	.42	.10	.71	.43	.23
22	.70	.35	.95	.66	.48	52	.47	.15	.77	.48	.28
21	.76	.41	43.01	.72	.54	51	.52	.21	.82	.53	.34
20	.81	.46	.06	.77	.59	50	.58	.26	.87	.58	.39
19	.87	.52	.12	.83	.65	49	.63	.31	.93	.64	.44
18	.93	.58	.17	.89	.71	48	.68	.36	.98	.69	.49
17	.98	.63	.23	.94	.76	47	.73	.41	47.03	.74	.54
16	46.04	.69	.29	42.00	.82	46	.78	.47	.08	.79	.60
15	.09	.74	.34	.06	.87	45	.83	.52	.14	.85	.65
14	.15	.80	.40	.11	.93	44	.89	.57	.19	.90	.70
13	.20	.86	.45	.17	.98	43	.94	.62	.24	.95	.75
12	.26	.91	.51	.22	41.04	42	.99	.68	.29	46.01	.81
11	.31	.97	.57	.28	.09	41	50.04	.73	.34	.06	.86
10	.37	45.03	.62	.33	.15	40	.09	.78	.40	.11	.91
09	.43	.08	.68	.39	.20	39	.14	.83	.45	.16	.96
08	.49	.14	.74	.44	.26	38	.19	.88	.50	.21	45.02
07	.54	.19	.79	.50	.31	37	.24	.94	.55	.27	.07
06	.59	.25	.85	.56	.37	36	.30	.99	.60	.32	.12
05	.65	.30	.90	.61	.42	35	.35	49.04	.66	.37	.17
04	.70	.36	.96	.67	.48	34	.40	.09	.71	.42	.22
03	.76	.42	44.02	.72	.53	33	.45	.14	.76	.47	.27
02	.81	.47	.07	.78	.59	32	.50	.19	.81	.53	.33
01	.87	.53	.13	.83	.64	31	.55	.25	.86	.58	.38
00	.92	.58	.18	.89	.70	30	.60	.30	.92	.63	.43
0.9399	.98	.64	.23	.94	.75	29	.65	.35	.97	.68	.48
98	47.03	.69	.29	43.00	.81	28	.70	.40	48.02	.73	.53
97	.09	.74	.34	.05	.86	27	.75	.45	.07	.79	.59
96	.14	.80	.40	.11	.92	26	.81	.50	.12	.84	.64
95	.19	.85	.45	.16	.97	25	.86	.56	.17	.89	.69
94	.25	.91	.51	.22	42.03	24	.91	.60	.22	.94	.74
93	.30	.96	.56	.27	.08	23	.96	.65	.28	.99	.79
92	.35	46.01	.62	.33	.14	22	51.01	.70	.33	47.05	.84
91	.41	.07	.67	.38	.19	21	.06	.75	.38	.10	.90
90	.46	.12	.73	.44	.24	20	.11	.80	.43	.15	.95
89	.52	.18	.78	.49	.30	19	.16	.85	.48	.20	46.00
88	.57	.23	.84	.55	.35	18	.21	.90	.53	.25	.05
87	.62	.29	.89	.60	.41	17	.26	.95	.58	.30	.10
86	.68	.34	.95	.65	.46	16	.31	50.00	.63	.35	.15
85	.73	.39	45.00	.71	.52	15	.36	.05	.68	.40	.20
84	.78	.45	.05	.77	.57	14	.41	.10	.73	.45	.26
83	.84	.50	.11	.82	.63	13	.46	.16	.79	.50	.31
82	.89	.56	.16	.87	.68	12	.51	.21	.84	.55	.36
81	.95	.61	.22	.93	.73	11	.56	.26	.89	.60	.41
80	48.00	.67	.27	.98	.79	10	.61	.31	.94	.65	.46
79	.05	.72	.32	44.04	.84	09	.66	.36	.99	.71	.51
78	.11	.77	.38	.09	.90	08	.71	.41	49.04	.76	.56
77	.16	.83	.43	.15	.95	07	.76	.46	.09	.81	.61
76	.21	.88	.48	.20	43.01	06	.81	.51	.14	.86	.66
75	.26	.94	.54	.25	.06	05	.86	.56	.19	.91	.71
74	.32	.99	.59	.31	.11	04	.91	.61	.24	.96	.77
73	.37	47.04	.65	.36	.17	03	.96	.66	.29	48.01	.82
72	.42	.10	.70	.41	.22	02	52.01	.71	.34	.06	.87
71	.48	.15	.75	.47	.27	01	.08	.76	.39	.11	.92
70	.53	.20	.81	.52	.33	00	.11	.81	.44	.16	.97

19 Percentages by volume at 60°F of ethyl alcohol corresponding to apparent specific gravities at various temperatures.—Continued.

APPARENT SPECIFIC GRAVITY	15.56	20/20	25/25	30/30	35/35	APPARENT SPECIFIC GRAVITY	15.56	20/20	25/25	30/30	35/35
15.56	15.56					15.56	15.56				
0.9300	52.11	50.81	49.44	48.16	46.97	0.9230	55.52	54.24	52.88	51.61	50.41
0.9290	16	.86	.49	.21	47.02	29	.57	.29	.03	.06	.46
98	.21	.91	.54	.26	.07	28	.62	.33	.98	.71	.51
97	.26	.96	.59	.31	.12	27	.67	.38	53.03	.75	.56
96	.31	51.01	.64	.36	.17	26	.71	.43	.08	.80	.60
95	.36	.06	.69	.41	.22	25	.76	.48	.12	.85	.65
94	.41	.11	.74	.46	.27	24	.81	.53	.17	.90	.70
93	.46	.16	.79	.51	.32	23	.86	.57	.22	.95	.75
92	.51	.21	.84	.56	.37	22	.90	.62	.27	52.00	.80
91	.56	.26	.89	.61	.42	21	.95	.67	.31	.04	.85
90	.61	.31	.94	.66	.47	20	56.00	.72	.36	.09	.80
89	.66	.36	.99	.71	.52	19	.05	.77	.41	.14	.94
88	.71	.41	50.04	.76	.57	18	.09	.81	.46	.19	.99
87	.76	.46	.09	.81	.62	17	.14	.86	.50	.23	51.04
86	.81	.50	.14	.86	.67	16	.19	.91	.55	.28	.09
85	.86	.55	.19	.91	.72	15	.24	.96	.60	.33	.13
84	.91	.60	.24	.96	.77	14	.28	55.00	.65	.38	.18
83	.96	.65	.29	49.01	.82	13	.33	.05	.70	.43	.23
82	53.00	.70	.34	.06	.87	12	.38	.10	.74	.47	.27
81	.05	.75	.39	.11	.92	11	.43	.15	.79	.52	.32
80	.10	.80	.44	.16	.97	10	.47	.19	.84	.57	.37
79	.15	.85	.49	.21	48.02	9	.52	.24	.89	.62	.42
78	.20	.90	.54	.26	.07	08	.57	.29	.93	.67	.46
77	.25	.95	.59	.31	.12	07	.62	.34	.98	.71	.51
76	.30	52.00	.64	.36	.17	06	.66	.38	54.03	.76	.56
75	.35	.05	.68	.41	.22	05	.71	.43	.08	.81	.61
74	.40	.10	.73	.46	.27	04	.76	.48	.12	.86	.65
73	.45	.15	.78	.51	.32	03	.81	.53	.17	.90	.70
72	.50	.20	.83	.56	.37	02	.85	.57	.22	.95	.75
71	.54	.25	.88	.61	.42	01	.90	.62	.26	53.00	.80
70	.59	.29	.93	.66	.47	00	.95	.67	.31	.05	.84
69	.64	.34	.98	.71	.52	0.9169	57.00	.71	.36	.09	.89
68	.69	.39	51.03	.76	.57	98	.04	.76	.41	.14	.94
67	.74	.44	.08	.81	.62	97	.09	.81	.45	.19	.99
66	.79	.49	.13	.86	.67	96	.13	.86	.50	.23	52.03
65	.84	.54	.18	.91	.71	95	.18	.90	.55	.28	.08
64	.89	.59	.23	.96	.76	94	.23	.95	.59	.33	.13
63	.94	.64	.27	50.00	.81	93	.27	56.00	.64	.37	.17
62	.99	.69	.32	.05	.86	92	.32	.04	.69	.42	.22
61	54.03	.74	.37	.10	.91	91	.37	.09	.74	.47	.27
60	.08	.79	.42	.15	.96	90	.41	.14	.78	.51	.32
59	.13	.84	.47	.20	49.01	89	.46	.18	.83	.56	.36
58	.18	.89	.52	.25	.06	88	.51	.23	.88	.61	.41
57	.23	.93	.57	.30	.11	87	.55	.28	.92	.65	.46
56	.28	.98	.62	.35	.15	86	.60	.32	.97	.70	.50
55	.32	53.03	.67	.40	.20	85	.65	.37	55.02	.75	.55
54	.37	.08	.72	.44	.25	84	.69	.42	.07	.79	.60
53	.42	.13	.76	.49	.30	83	.74	.46	.11	.84	.65
52	.47	.18	.81	.54	.35	82	.79	.51	.16	.89	.69
51	.52	.22	.86	.59	.40	81	.83	.56	.21	.93	.74
50	.57	.27	.91	.64	.44	80	.88	.60	.25	.98	.79
49	.61	.32	.96	.69	.49	79	.93	.65	.30	54.03	.83
48	.66	.37	52.01	.74	.54	78	.97	.70	.35	.07	.88
47	.71	.42	.06	.79	.59	77	58.02	.74	.39	.12	.93
46	.76	.47	.11	.83	.64	76	.06	.79	.44	.17	.98
45	.81	.52	.16	.88	.69	75	.11	.84	.49	.21	53.02
44	.86	.57	.20	.93	.73	74	.16	.88	.53	.26	.07
43	.90	.61	.25	.98	.78	73	.20	.93	.58	.31	.12
42	.95	.66	.30	51.03	.83	72	.25	.97	.63	.36	.16
41	55.00	.71	.35	.08	.88	71	.29	57.02	.67	.40	.21
40	.05	.76	.40	.13	.93	70	.34	.07	.72	.45	.26
39	.10	.81	.45	.17	.98	69	.38	.11	.77	.50	.30
38	.14	.85	.50	.22	50.02	68	.43	.16	.81	.54	.35
37	.19	.90	.54	.27	.07	67	.47	.21	.86	.59	.40
36	.24	.95	.59	.32	.12	66	.52	.25	.91	.64	.44
35	.29	54.00	.64	.37	.17	65	.57	.30	.95	.68	.49
34	.33	.06	.69	.42	.22	64	.61	.35	56.00	.73	.53
33	.38	.09	.74	.46	.27	63	.66	.39	.05	.78	.58
32	.43	.14	.79	.51	.31	62	.70	.44	.09	.82	.63
31	.48	.19	.83	.56	.36	61	.75	.48	.14	.87	.67
30	.52	.24	.88	.61	.41	60	.79	.53	.18	.92	.72

REFERENCE TABLES

XLII

Percentages by volume at 60°F of ethyl alcohol corresponding to apparent specific gravities at various temperatures.—Continued.

APPARENT SPECIFIC GRAVITY	15.56						APPARENT SPECIFIC GRAVITY	15.56					
	15.56	20/20	25/25	30/30	35/35			15.56	20/20	25/25	30/30	35/35	
0.9160	58.79	57.53	56.18	54.92	53.72		0.9090	61.92	60.68	59.36	58.11	56.93	
59	.84	.58	.23	.06	.07		80	.96	.72	.49	.18	.97	
58	.89	.62	.28	.50	.81		88	62.01	.77	.45	.20	57.02	
57	.93	.67	.32	.06	.86		87	.05	.81	.49	.24	.06	
56	.98	.71	.37	.10	.91		86	.10	.86	.54	.29	.11	
55	59.02	.76	.41	.15	.95		85	.14	.90	.58	.33	.15	
54	.07	.81	.46	.19	64.00		84	.18	.94	.63	.38	.19	
53	.11	.85	.51	.24	.05		83	.23	.99	.67	.42	.24	
52	.16	.90	.55	.29	.09		82	.27	61.03	.71	.46	.28	
51	.20	.94	.60	.33	.14		81	.31	.08	.76	.51	.33	
50	.25	.99	.65	.38	.18		80	.36	.12	.80	.55	.37	
49	.29	58.03	.69	.42	.23		79	.40	.17	.85	.60	.42	
48	.34	.08	.74	.47	.28		78	.45	.21	.89	.64	.46	
47	.38	.13	.78	.52	.32		77	.49	.25	.94	.69	.50	
46	.43	.17	.83	.56	.37		76	.53	.30	.98	.73	.55	
45	.47	.22	.88	.61	.41		75	.58	.34	60.03	.77	.59	
44	.52	.26	.92	.65	.46		74	.62	.39	.07	.82	.64	
43	.56	.31	.97	.70	.51		73	.66	.43	.11	.86	.68	
42	.61	.35	57.01	.75	.55		72	.71	.47	.16	.91	.73	
41	.65	.40	.06	.79	.60		71	.75	.52	.20	.95	.77	
40	.70	.44	.10	.84	.65		70	.79	.56	.25	59.00	.81	
39	.74	.49	.15	.88	.69		69	.84	.60	.29	.85	.86	
38	.79	.53	.20	.93	.74		68	.88	.65	.33	.08	.90	
37	.83	.58	.24	.98	.78		67	.93	.69	.38	.13	.95	
36	.88	.62	.29	56.02	.83		66	.97	.74	.42	.17	.99	
35	.92	.67	.33	.07	.88		65	63.01	.78	.46	.21	58.04	
34	.97	.71	.38	.11	.92		64	.06	.82	.51	.26	.08	
33	60.01	.76	.42	.16	.97		63	.10	.87	.55	.30	.12	
32	.06	.80	.47	.21	55.01		62	.14	.91	.60	.35	.17	
31	.10	.85	.51	.25	.06		61	.19	.96	.64	.39	.21	
30	.15	.89	.56	.30	.11		60	.23	62.00	.68	.43	.26	
29	.19	.94	.60	.34	.15		59	.27	.04	.73	.48	.30	
28	.24	.98	.65	.39	.20		58	.32	.09	.77	.52	.34	
27	.28	59.03	.70	.44	.24		57	.36	.13	.82	.57	.39	
26	.33	.07	.74	.48	.29		56	.40	.17	.86	.61	.43	
25	.37	.12	.79	.53	.33		55	.45	.22	.90	.65	.48	
24	.42	.16	.83	.57	.38		54	.49	.26	.95	.70	.52	
23	.46	.21	.88	.62	.42		53	.53	.30	.99	.74	.56	
22	.50	.25	.92	.67	.47		52	.58	.35	61.03	.79	.61	
21	.55	.30	.97	.71	.52		51	.62	.39	.08	.83	.65	
20	.59	.34	58.01	.76	.56		50	.66	.43	.12	.87	.70	
19	.64	.39	.06	.80	.61		49	.71	.48	.16	.92	.74	
18	.68	.43	.10	.85	.65		48	.75	.52	.21	.96	.78	
17	.73	.48	.15	.89	.70		47	.79	.56	.25	60.00	.82	
16	.77	.52	.19	.94	.74		46	.84	.60	.29	.05	.87	
15	.82	.57	.24	.99	.79		45	.88	.65	.34	.09	.92	
14	.86	.61	.28	57.03	.84		44	.92	.69	.38	.14	.96	
13	.91	.66	.33	.08	.88		43	.97	.73	.42	.18	59.00	
12	.95	.70	.37	.12	.93		42	64.01	.78	.47	.22	.05	
11	61.00	.75	.42	.17	.97		41	.05	.82	.51	.27	.09	
10	.04	.79	.46	.21	56.02		40	.09	.86	.55	.31	.13	
09	.08	.84	.51	.26	.06		39	.14	.91	.60	.35	.18	
08	.13	.88	.55	.30	.11		38	.18	.95	.64	.40	.22	
07	.17	.92	.60	.35	.15		37	.22	.99	.68	.44	.27	
06	.22	.97	.64	.39	.20		36	.27	63.04	.73	.48	.31	
05	.26	60.01	.69	.44	.25		35	.31	.08	.77	.53	.35	
04	.30	.06	.73	.48	.29		34	.35	.12	.81	.57	.40	
03	.35	.10	.78	.53	.34		33	.40	.17	.86	.62	.44	
02	.39	.15	.82	.57	.38		32	.44	.21	.90	.66	.48	
01	.44	.19	.87	.62	.43		31	.48	.25	.94	.70	.53	
00	.48	.24	.91	.66	.47		30	.53	.30	.99	.75	.57	
0.9090	.52	.38	.06	.71	.52		29	.57	.34	62.03	.79	.61	
98	.57	.33	59.00	.75	.56		28	.61	.38	.07	.83	.66	
97	.61	.37	.04	.80	.61		27	.66	.43	.12	.88	.70	
96	.66	.41	.09	.84	.65		26	.70	.47	.16	.92	.74	
95	.70	.46	.13	.89	.70		25	.74	.51	.20	.97	.79	
94	.74	.50	.18	.93	.75		24	.78	.56	.24	61.01	.83	
93	.79	.55	.22	.98	.79		23	.83	.60	.29	.05	.87	
92	.83	.59	.27	58.02	.84		22	.87	.64	.33	.09	.92	
91	.88	.64	.31	.07	.88		21	.91	.69	.37	.14	.96	
90	.92	.68	.36	.11	.93		20	.96	.73	.42	.18	60.00	



19 Percentages by volume at 60°F of ethyl alcohol corresponding to apparent specific gravities at various temperatures.—Continued.

APPARENT SPECIFIC GRAVITY	15.56	20.20	25.25	30.30	35.35	APPARENT SPECIFIC GRAVITY	15.56	20.20	25.25	30.30	35.35
	15.56						15.56				
0.9020	64.06	63.73	62.42	61.18	60.00	0.8950	67.90	66.69	65.39	64.16	62.99
19	65.00	.77	.46	.22	.05	49	.94	.73	.44	.21	63.03
18	.04	.82	.50	.27	.09	48	.98	.77	.48	.25	.08
17	.09	.86	.55	.31	.13	47	68.02	.81	.52	.29	.12
16	.13	.90	.59	.35	.18	46	.07	.85	.56	.33	.16
15	.17	.94	.63	.40	.22	45	.11	.90	.60	.37	.20
14	.21	.99	.67	.44	.26	44	.15	.94	.64	.42	.24
13	.26	64.03	.72	.48	.30	43	.19	.98	.69	.46	.28
12	.30	.07	.76	.53	.35	42	.23	67.02	.73	.50	.33
11	.34	.11	.80	.57	.39	41	.27	.06	.77	.54	.37
10	.38	.16	.85	.61	.43	40	.31	.10	.81	.58	.41
09	.43	.20	.89	.66	.48	39	.35	.15	.85	.63	.45
08	.47	.24	.93	.70	.52	38	.39	.19	.90	.67	.49
07	.51	.28	.97	.74	.56	37	.43	.23	.94	.71	.54
06	.55	.32	63.02	.78	.61	36	.47	.27	.98	.75	.58
05	.60	.37	.06	.83	.65	35	.52	.31	66.02	.79	.62
04	.64	.41	.10	.87	.69	34	.56	.35	.06	.84	.66
03	.68	.45	.15	.91	.73	33	.60	.39	.10	.88	.70
02	.72	.50	.19	.96	.78	32	.64	.43	.15	.92	.74
01	.77	.54	.23	62.00	.82	31	.68	.47	.19	.96	.79
00	.81	.58	.27	.04	.86	30	.72	.52	.23	65.00	.83
0.8999	.85	.62	.32	.09	.91	29	.76	.56	.27	.05	.87
98	.89	.67	.36	.13	.95	28	.80	.60	.31	.09	.91
97	.94	.71	.40	.17	.99	27	.84	.64	.35	.13	.95
96	.98	.75	.44	.21	61.03	26	.89	.68	.39	.17	64.00
95	66.02	.79	.49	.26	.08	25	.93	.72	.44	.21	.04
94	.06	.84	.53	.30	.12	24	.97	.76	.48	.25	.08
93	.10	.88	.57	.34	.16	23	69.01	.80	.52	.29	.12
92	.15	.92	.62	.38	.21	22	.05	.84	.56	.34	.16
91	.19	.96	.66	.43	.25	21	.09	.89	.60	.38	.21
90	.23	65.01	.70	.47	.29	20	.13	.93	.64	.42	.25
89	.27	.05	.74	.51	.33	19	.17	.97	.68	.46	.29
88	.31	.09	.79	.55	.38	18	.21	68.01	.73	.50	.33
87	.36	.13	.83	.60	.42	17	.25	.05	.77	.54	.37
86	.40	.18	.87	.64	.46	16	.29	.09	.81	.59	.41
85	.44	.22	.91	.68	.50	15	.33	.13	.85	.63	.46
84	.48	.26	.96	.72	.55	14	.37	.17	.89	.67	.50
83	.52	.30	64.00	.77	.59	13	.41	.21	.93	.71	.54
82	.56	.35	.01	.81	.63	12	.46	.26	.98	.75	.58
81	.61	.39	.08	.85	.68	11	.50	.30	67.02	.79	.62
80	.65	.43	.13	.89	.72	10	.54	.34	.06	.83	.67
79	.69	.47	.17	.94	.76	09	.58	.38	.10	.88	.71
78	.73	.51	.21	.98	.80	08	.62	.42	.14	.92	.75
77	.77	.56	.25	63.02	.85	07	.66	.46	.18	.96	.79
76	.82	.60	.30	.06	.89	06	.70	.50	.22	66.00	.83
75	.86	.64	.34	.11	.93	05	.74	.54	.26	.04	.87
74	.90	.68	.38	.15	.97	04	.78	.58	.30	.08	.92
73	.94	.72	.42	.19	62.02	03	.82	.62	.34	.12	.96
72	.98	.77	.47	.23	.06	02	.86	.67	.39	.17	65.00
71	67.03	.81	.51	.28	.10	01	.90	.71	.43	.21	.04
70	.07	.85	.55	.32	.14	00	.94	.75	.47	.25	.08
69	.11	.89	.59	.36	.19	0.8999	.98	.79	.51	.29	.12
68	.15	.94	.64	.40	.23	98	70.02	.83	.55	.33	.17
67	.19	.98	.68	.44	.27	97	.06	.87	.59	.37	.21
66	.23	66.02	.72	.49	.31	96	.10	.91	.63	.41	.25
65	.28	.06	.76	.53	.36	95	.14	.95	.67	.45	.29
64	.32	.10	.81	.57	.40	94	.18	.99	.71	.50	.33
63	.36	.15	.85	.61	.44	93	.22	69.03	.75	.54	.37
62	.40	.19	.89	.66	.48	92	.27	.07	.80	.58	.41
61	.44	.23	.93	.70	.53	91	.31	.11	.84	.62	.45
60	.48	.27	.97	.74	.57	90	.35	.15	.88	.66	.50
59	.53	.31	65.02	.78	.61	89	.39	.19	.92	.70	.54
58	.57	.36	.06	.83	.65	88	.43	.23	.96	.74	.58
57	.61	.40	.10	.87	.69	87	.47	.27	68.00	.79	.62
56	.65	.44	.14	.91	.74	86	.51	.32	.04	.83	.66
55	.69	.48	.18	.95	.78	85	.55	.36	.08	.87	.70
54	.73	.52	.23	64.00	.82	84	.59	.40	.12	.91	.74
53	.78	.56	.27	.04	.86	83	.63	.44	.16	.95	.79
52	.82	.60	.31	.08	.90	82	.67	.48	.20	.99	.83
51	.86	.65	.35	.12	.95	81	.71	.52	.24	67.03	.87
50	.90	.69	.39	.16	.99	80	.75	.56	.28	.07	.91

REFERENCE TABLES

XLIII

Percentages by volume at 60°F of ethyl alcohol corresponding to apparent specific gravities at various temperatures.—Continued. 19

APPARENT SPECIFIC GRAVITY	15.56	20/20	25/25	30/30	35/35	APPARENT SPECIFIC GRAVITY	15.56	20/20	25/25	30/30	35/35
	15.56						15.56				
0.8980	70.75	69.56	68.28	67.07	65.91	0.8810	73.50	72.34	71.09	69.89	68.74
79	.79	.60	.33	.11	.95	09	.54	.38	.13	.93	.78
78	.83	.64	.37	.15	.99	08	.58	.42	.16	.97	.83
77	.87	.68	.41	.20	66.03	07	.62	.46	.20	70.01	.86
76	.91	.72	.45	.24	.07	06	.66	.50	.24	.05	.90
75	.95	.76	.49	.28	.11	05	.70	.53	.28	.09	.94
74	.99	.80	.53	.32	.16	04	.74	.57	.32	.13	.98
73	71.03	.84	.57	.36	.20	03	.78	.61	.36	.17	69.02
72	.07	.88	.61	.40	.24	02	.81	.65	.40	.06	.06
71	.11	.92	.65	.44	.28	01	.85	.69	.44	.25	.10
70	.15	.96	.68	.48	.32	00	.89	.73	.48	.29	.14
69	.19	70.00	.73	.52	.36	0.8790	.93	.77	.52	.33	.18
68	.23	.04	.77	.56	.40	98	.97	.81	.56	.37	.22
67	.27	.08	.81	.60	.44	97	74.01	.85	.60	.41	.26
66	.31	.12	.85	.64	.48	96	.05	.88	.64	.44	.30
65	.35	.16	.89	.68	.52	95	.08	.92	.67	.48	.34
64	.38	.20	.93	.72	.56	94	.12	.96	.71	.52	.38
63	.42	.24	.98	.76	.60	93	.16	73.00	.75	.56	.42
62	.46	.28	69.02	.80	.64	92	.20	.04	.79	.60	.45
61	.50	.32	.06	.85	.69	91	.24	.08	.83	.64	.49
60	.54	.36	.10	.89	.73	90	.28	.12	.87	.68	.53
59	.58	.40	.14	.93	.77	89	.32	.16	.91	.72	.57
58	.62	.44	.18	.97	.81	88	.36	.19	.95	.76	.61
57	.66	.48	.22	68.01	.85	87	.39	.23	.99	.80	.65
56	.70	.52	.26	.05	.89	86	.43	.27	72.03	.84	.69
55	.74	.56	.30	.09	.93	85	.47	.31	.07	.88	.73
54	.78	.60	.34	.13	.97	84	.51	.35	.11	.92	.77
53	.82	.64	.38	.17	67.01	83	.55	.39	.15	.96	.81
52	.86	.68	.42	.21	.05	82	.59	.43	.18	71.00	.85
51	.90	.72	.46	.25	.09	81	.63	.47	.22	.04	.89
50	.94	.76	.50	.29	.13	80	.66	.50	.26	.07	.93
49	.98	.80	.54	.33	.17	79	.70	.54	.30	.11	.97
48	72.02	.84	.58	.37	.21	78	.74	.58	.34	.15	70.01
47	.06	.88	.62	.41	.25	77	.78	.62	.38	.19	.05
46	.10	.92	.66	.45	.29	76	.82	.66	.42	.23	.09
45	.14	.96	.70	.49	.33	75	.86	.70	.46	.27	.13
44	.18	71.00	.74	.53	.38	74	.90	.74	.49	.31	.16
43	.22	.04	.78	.57	.42	73	.93	.78	.52	.35	.20
42	.25	.08	.82	.61	.46	72	.97	.81	.57	.39	.24
41	.29	.12	.86	.65	.50	71	75.01	.85	.61	.42	.28
40	.33	.16	.90	.69	.54	70	.05	.89	.65	.46	.32
39	.37	.20	.94	.73	.58	69	.09	.93	.69	.50	.36
38	.41	.24	.98	.77	.62	68	.13	.97	.73	.54	.40
37	.45	.27	70.02	.81	.66	67	.16	74.01	.77	.58	.44
36	.49	.31	.06	.85	.70	66	.20	.05	.81	.62	.48
35	.53	.35	.10	.89	.74	65	.24	.08	.84	.66	.52
34	.57	.39	.13	.93	.78	64	.28	.12	.88	.70	.56
33	.61	.43	.17	.97	.82	63	.32	.16	.92	.74	.60
32	.65	.47	.21	69.01	.86	62	.35	.20	.96	.77	.64
31	.69	.51	.25	.05	.90	61	.39	.24	73.00	.81	.67
30	.73	.55	.29	.09	.94	60	.43	.28	.04	.85	.71
29	.76	.59	.33	.13	.98	59	.47	.32	.08	.89	.75
28	.80	.63	.37	.17	68.02	58	.51	.35	.12	.93	.79
27	.84	.67	.41	.21	.06	57	.54	.39	.15	.97	.82
26	.88	.71	.45	.25	.10	56	.58	.43	.19	72.01	.87
25	.92	.75	.49	.29	.14	55	.62	.47	.23	.05	.91
24	.96	.79	.53	.33	.18	54	.66	.51	.27	.08	.95
23	73.00	.83	.57	.37	.22	53	.70	.55	.31	.12	.99
22	.04	.87	.61	.41	.26	52	.73	.58	.35	.16	71.03
21	.08	.91	.65	.45	.30	51	.77	.62	.38	.20	.07
20	.12	.95	.69	.49	.34	50	.81	.66	.42	.24	.10
19	.16	.99	.73	.53	.38	49	.85	.70	.46	.28	.14
18	.19	72.03	.77	.57	.42	48	.89	.74	.50	.32	.18
17	.23	.07	.81	.61	.46	47	.92	.77	.54	.36	.22
16	.27	.10	.85	.65	.50	46	.96	.81	.58	.39	.26
15	.31	.14	.89	.69	.54	45	76.00	.85	.62	.43	.30
14	.35	.18	.93	.73	.58	44	.04	.89	.65	.47	.34
13	.39	.22	.97	.77	.62	43	.07	.93	.69	.51	.38
12	.43	.26	71.01	.81	.66	42	.11	.97	.73	.55	.41
11	.47	.30	.06	.85	.70	41	.15	75.00	.77	.59	.45
10	.50	.34	.09	.89	.74	40	.19	.04	.81	.63	.49

19 Percentages by volume at 60°F of ethyl alcohol corresponding to apparent specific gravities at various temperatures.—Continued.

APPARENT SPECIFIC GRAVITY	15.56	20/20	25/25	30/30	35/35	APPARENT SPECIFIC GRAVITY	15.56	20/20	25/25	30/30	35/35
	15.56						15.56				
0.8740	76.19	75.04	73.81	72.63	71.49	0.8670	78.78	77.66	76.45	75.29	74.17
39	.22	.08	.85	.06	.53	69	.82	.70	.49	.33	.21
38	.26	.12	.88	.70	.57	68	.85	.73	.53	.37	.24
37	.30	.16	.92	.74	.61	67	.89	.77	.56	.40	.28
36	.34	.19	.96	.78	.65	66	.93	.81	.60	.44	.32
35	.37	.23	74.00	.82	.69	65	.96	.84	.64	.48	.36
34	.41	.27	.04	.86	.72	64	79.00	.88	.68	.51	.39
33	.45	.31	.08	.90	.76	63	.04	.92	.71	.55	.43
32	.49	.35	.11	.93	.80	62	.07	.96	.75	.59	.47
31	.52	.38	.15	.97	.84	61	.11	.99	.79	.63	.51
30	.56	.42	.19	73.01	.88	60	.14	78.03	.82	.66	.55
29	.60	.46	.23	.05	.92	59	.18	.07	.86	.70	.58
28	.64	.50	.27	.09	.96	58	.22	.10	.90	.74	.62
27	.67	.54	.31	.13	72.00	57	.25	.14	.94	.78	.66
26	.71	.57	.34	.16	.03	56	.29	.17	.97	.81	.70
25	.75	.61	.38	.20	.07	55	.32	.21	77.01	.85	.73
24	.79	.65	.42	.24	.11	54	.36	.25	.05	.89	.77
23	.82	.69	.46	.28	.15	53	.40	.28	.08	.93	.81
22	.86	.73	.50	.32	.19	52	.43	.32	.12	.96	.85
21	.90	.76	.53	.35	.23	51	.47	.36	.16	76.00	.88
20	.94	.80	.57	.39	.27	50	.51	.39	.19	.04	.92
19	.97	.84	.61	.43	.30	49	.54	.43	.23	.07	.96
18	77.01	.88	.65	.47	.34	48	.58	.47	.27	.11	75.00
17	.05	.91	.69	.51	.38	47	.61	.50	.30	.15	.03
16	.09	.95	.73	.55	.42	46	.65	.54	.34	.19	.07
15	.12	.99	.76	.58	.46	45	.69	.57	.38	.22	.11
14	.16	76.03	.80	.62	.50	44	.72	.61	.41	.26	.15
13	.20	.06	.84	.66	.53	43	.76	.65	.45	.30	.18
12	.24	.10	.88	.70	.57	42	.79	.68	.49	.33	.22
11	.27	.11	.92	.74	.61	41	.83	.72	.52	.37	.26
10	.31	.18	.95	.77	.65	40	.87	.76	.56	.41	.29
09	.34	.22	.99	.81	.69	39	.90	.79	.60	.44	.33
08	.38	.25	75.05	.85	.73	38	.94	.83	.63	.48	.37
07	.42	.29	.07	.89	.77	37	.97	.86	.67	.52	.41
06	.46	.33	.10	.93	.80	36	80.01	.90	.71	.56	.44
05	.49	.37	.14	.97	.84	35	.05	.94	.74	.59	.48
04	.53	.40	.18	74.00	.88	34	.08	.97	.78	.63	.52
03	.57	.44	.22	.04	.92	33	.12	79.01	.82	.67	.56
02	.60	.48	.25	.08	.96	32	.15	.05	.85	.70	.59
01	.64	.52	.29	.12	72.00	31	.19	.08	.89	.74	.63
00	.68	.55	.33	.16	.03	30	.22	.12	.93	.78	.67
0.8699	.71	.59	.37	.19	.07	29	.26	.16	.96	.81	.71
98	.75	.63	.40	.21	.11	28	.30	.19	78.00	.85	.74
97	.79	.66	.44	.27	.15	27	.33	.23	.04	.89	.78
96	.83	.70	.48	.31	.19	26	.37	.26	.07	.93	.82
95	.86	.74	.52	.35	.22	25	.40	.30	.11	.96	.85
94	.90	.78	.55	.38	.26	24	.44	.34	.14	77.00	.89
93	.94	.81	.59	.42	.30	23	.47	.37	.18	.04	.93
92	.97	.85	.63	.46	.34	22	.51	.41	.22	.07	.97
91	78.01	.89	.67	.50	.38	21	.55	.45	.25	.11	76.00
90	.05	.92	.70	.54	.41	20	.58	.48	.29	.15	.04
89	.08	.96	.74	.57	.45	19	.62	.52	.33	.18	.08
88	.12	77.00	.78	.61	.49	18	.65	.55	.36	.22	.11
87	.16	.09	.82	.65	.53	17	.69	.59	.40	.26	.15
86	.19	.07	.85	.69	.56	16	.72	.63	.43	.29	.19
85	.23	.11	.89	.73	.60	15	.76	.66	.47	.33	.23
84	.27	.14	.93	.76	.64	14	.80	.70	.51	.36	.26
83	.30	.18	.97	.80	.68	13	.83	.73	.54	.40	.30
82	.34	.22	76.00	.84	.72	12	.87	.77	.58	.44	.34
81	.38	.26	.04	.88	.75	11	.90	.80	.62	.47	.37
80	.41	.29	.08	.92	.79	10	.94	.84	.65	.51	.41
79	.45	.33	.12	.96	.83	09	.97	.88	.69	.55	.45
78	.49	.37	.15	.99	.87	08	81.01	.91	.72	.58	.48
77	.52	.40	.19	75.03	.91	07	.05	.95	.76	.62	.52
76	.56	.44	.23	.07	.94	06	.08	.98	.80	.66	.56
75	.60	.48	.26	.10	.98	05	.12	80.02	.83	.69	.59
74	.63	.51	.30	.14	74.02	04	.15	.05	.87	.73	.63
73	.67	.55	.34	.18	.06	03	.19	.09	.91	.77	.67
72	.71	.59	.38	.22	.09	02	.22	.13	.94	.80	.70
71	.74	.62	.41	.25	.13	01	.26	.16	.98	.84	.74
70	.78	.66	.45	.29	.17	00	.29	.20	79.01	.88	.78

REFERENCE TABLES

XLII

Percentages by volume at 60°F of ethyl alcohol corresponding to apparent specific gravities at various temperatures.—Continued.

APPARENT SPECIFIC GRAVITY	15.56						APPARENT SPECIFIC GRAVITY	15.56					
	15.56	20/20	25/25	30/30	35/35			15.56	20/20	25/25	30/30	35/35	
0.8400	81.29	80.20	79.01	77.88	76.78		0.8530	83.73	82.66	81.50	80.38	79.30	
0.8509	81.33	.23	.05	.91	.82			.77	.69	.54	.42	.34	
98	.36	.27	.09	.95	.85		29	.80	.73	.57	.45	.38	
97	.40	.30	.12	.99	.89		27	.84	.76	.61	.49	.41	
96	.43	.34	.16	.78	.02	.93	26	.87	.80	.64	.52	.45	
95	.47	.38	.19	.06	.36		25	.90	.83	.68	.56	.48	
94	.51	.41	.23	.09	.77	.00	24	.94	.87	.71	.59	.52	
93	.54	.45	.27	.13	.04		23	.97	.90	.75	.63	.55	
92	.58	.48	.30	.17	.07		22	84.01	.94	.78	.66	.59	
91	.61	.52	.34	.20	.11		21	.04	.97	.82	.70	.62	
90	.65	.55	.37	.24	.14		20	.07	83.01	.85	.73	.66	
89	.68	.59	.41	.27	.18		19	.11	.04	.89	.77	.70	
88	.72	.62	.44	.31	.22		18	.14	.07	.92	.81	.73	
87	.75	.66	.48	.35	.25		17	.18	.11	.96	.84	.77	
86	.79	.69	.52	.38	.29		16	.21	.14	.99	.88	.80	
85	.82	.73	.55	.42	.33		15	.24	.18	82.03	.91	.84	
84	.86	.77	.59	.45	.36		14	.28	.21	.06	.95	.87	
83	.89	.80	.62	.49	.40		13	.31	.25	.10	.98	.91	
82	.93	.84	.66	.53	.43		12	.34	.28	.13	81.02	.94	
81	.96	.87	.70	.56	.47		11	.38	.32	.16	.05	.98	
80	82.00	.91	.73	.60	.51		10	.41	.35	.20	.09	80.01	
79	.03	.94	.77	.64	.54		09	.45	.39	.23	.12	.05	
78	.07	.98	.80	.67	.58		08	.48	.42	.27	.16	.08	
77	.10	81.01	.84	.71	.62		07	.51	.45	.30	.19	.12	
76	.14	.05	.87	.74	.65		06	.55	.49	.34	.23	.15	
75	.17	.08	.91	.78	.69		05	.58	.52	.37	.26	.19	
74	.21	.12	.95	.82	.72		04	.61	.56	.41	.30	.23	
73	.24	.16	.98	.85	.76		03	.65	.59	.44	.33	.26	
72	.28	.19	80.02	.89	.80		02	.68	.62	.47	.37	.30	
71	.31	.23	.05	.92	.83		01	.72	.66	.51	.40	.33	
70	.35	.26	.09	.96	.87		00	.75	.69	.54	.44	.37	
69	.38	.30	.12	79.00	.91		0.8499	.78	.73	.58	.47	.40	
68	.42	.34	.16	.03	.94		98	.82	.76	.61	.51	.44	
67	.45	.37	.20	.07	.98		97	.85	.79	.65	.54	.47	
66	.49	.40	.23	.10	78.01		96	.89	.83	.68	.57	.51	
65	.52	.44	.27	.14	.05		95	.92	.86	.71	.61	.54	
64	.56	.47	.30	.17	.09		94	.95	.90	.75	.64	.58	
63	.59	.51	.34	.21	.12		93	.99	.93	.78	.68	.61	
62	.63	.54	.37	.25	.16		92	85.02	.97	.82	.71	.65	
61	.66	.58	.41	.28	.19		91	.05	84.00	.85	.76	.68	
60	.70	.61	.44	.32	.23		90	.09	.03	.89	.78	.72	
59	.73	.65	.48	.35	.27		89	.12	.07	.92	.82	.75	
58	.77	.68	.51	.39	.30		88	.15	.10	.96	.85	.79	
57	.80	.72	.55	.42	.34		87	.18	.14	.99	.89	.82	
56	.84	.75	.59	.46	.37		86	.22	.17	83.02	.92	.86	
55	.87	.79	.62	.49	.41		85	.25	.20	.06	.96	.89	
54	.91	.82	.66	.53	.45		84	.28	.24	.09	.99	.93	
53	.94	.86	.69	.57	.48		83	.32	.27	.13	82.03	.96	
52	.98	.89	.73	.60	.52		82	.35	.31	.16	.06	81.00	
51	83.01	.93	.76	.64	.55		81	.38	.34	.20	.10	.03	
50	.04	.96	.80	.67	.59		80	.42	.37	.23	.13	.07	
49	.08	82.00	.83	.71	.63		79	.45	.41	.26	.17	.10	
48	.11	.03	.87	.74	.66		78	.48	.44	.30	.20	.14	
47	.15	.07	.90	.78	.70		77	.51	.47	.33	.24	.17	
46	.18	.10	.94	.81	.73		76	.55	.51	.37	.27	.21	
45	.22	.14	.98	.85	.77		75	.58	.54	.40	.30	.24	
44	.25	.17	81.01	.89	.81		74	.61	.57	.43	.34	.28	
43	.29	.21	.05	.92	.84		73	.65	.61	.47	.37	.31	
42	.32	.24	.08	.96	.88		72	.68	.64	.50	.41	.35	
41	.35	.28	.12	.99	.91		71	.71	.67	.54	.44	.38	
40	.39	.31	.15	80.03	.95		70	.75	.71	.57	.48	.42	
39	.42	.35	.19	.06	.99		69	.78	.74	.61	.51	.45	
38	.46	.38	.22	.10	79.02		68	.81	.78	.64	.55	.49	
37	.49	.42	.26	.13	.06		67	.84	.81	.67	.58	.52	
36	.53	.45	.29	.17	.09		66	.88	.84	.71	.62	.56	
35	.56	.49	.30	.20	.13		65	.91	.88	.74	.65	.59	
34	.59	.52	.36	.24	.16		64	.94	.91	.78	.69	.63	
33	.63	.55	.40	.28	.20		63	.98	.94	.81	.72	.66	
32	.66	.59	.43	.31	.23		62	86.01	.98	.85	.75	.70	
31	.70	.62	.47	.35	.27		61	.04	85.01	.88	.79	.73	
30	.73	.66	.50	.38	.30		60	.08	.04	.91	.82	.77	

19 Percentages by volume at 60°F of ethyl alcohol corresponding to apparent specific gravities at various temperatures.—Continued.

APPARENT SPECIFIC GRAVITY	15.56	20/20	25/25	30/30	35/35	APPARENT SPECIFIC GRAVITY	15.56	20/20	25/25	30/30	35/35
	15.56						15.56				
0.8460	86.08	85.04	83.91	82.82	81.77	0.8500	88.33	87.33	86.24	85.18	84.16
59	.11	.08	.95	.86	.80		.36	.36	.25	.22	.19
58	.14	.11	.98	.89	.84		.39	.39	.31	.25	.22
57	.17	.14	.84.02	.93	.87		.43	.43	.34	.28	.26
56	.21	.18	.05	.96	.91		.46	.46	.37	.31	.29
55	.24	.21	.08	88.00	.94		.49	.49	.40	.35	.32
54	.27	.24	.12	.03	.98		.52	.52	.44	.38	.36
53	.30	.28	.15	.06	82.01		.55	.55	.47	.41	.39
52	.34	.31	.18	.10	.04		.58	.58	.50	.45	.42
51	.37	.34	.22	.13	.08		.61	.62	.53	.48	.46
50	.40	.38	.25	.17	.11		.65	.65	.57	.51	.49
49	.43	.41	.29	.20	.15		.68	.68	.60	.54	.52
48	.47	.44	.32	.23	.18		.71	.71	.63	.58	.56
47	.50	.48	.35	.27	.22		.74	.74	.66	.61	.59
46	.53	.51	.39	.30	.25		.77	.78	.70	.64	.62
45	.57	.54	.42	.34	.28		.80	.81	.73	.68	.65
44	.60	.57	.45	.37	.32		.83	.84	.76	.71	.69
43	.63	.61	.49	.40	.35		.87	.87	.79	.74	.72
42	.66	.64	.52	.44	.39		.90	.90	.83	.77	.75
41	.70	.67	.55	.47	.42		.93	.94	.86	.81	.79
40	.73	.71	.59	.51	.46		.96	.97	.89	.84	.82
39	.76	.74	.62	.54	.49		.99	88.00	.92	.87	.85
38	.79	.77	.65	.57	.52		89.02	.03	.95	.90	.88
37	.83	.80	.69	.61	.56		.05	.06	.99	.94	.92
36	.86	.84	.72	.64	.59		.08	.09	87.02	.97	.95
35	.89	.87	.76	.68	.63		.11	.13	.05	86.00	.99
34	.92	.90	.79	.71	.66		.14	.16	.08	.04	85.02
33	.96	.94	.82	.74	.70		.18	.19	.11	.07	.05
32	.99	.97	.86	.78	.73		.21	.22	.15	.10	.08
31	87.02	86.00	.89	.81	.76		.24	.25	.18	.13	.12
30	.05	.03	.92	.85	.80		.27	.29	.21	.16	.15
29	.09	.07	.96	.88	.83		.30	.32	.24	.20	.18
28	.12	.10	.99	.91	.87		.33	.35	.27	.23	.22
27	.15	.13	85.02	.95	.90		.36	.38	.31	.26	.25
26	.18	.16	.06	.98	.93		.39	.41	.34	.29	.28
25	.22	.20	.09	84.02	.97		.42	.44	.37	.33	.31
24	.25	.23	.12	.05	83.00		.45	.47	.40	.36	.35
23	.28	.26	.16	.08	.04		.48	.50	.43	.39	.38
22	.31	.30	.19	.12	.07		.51	.54	.46	.42	.41
21	.34	.33	.22	.15	.11		.54	.57	.50	.45	.44
20	.38	.36	.25	.18	.14		.58	.60	.53	.49	.48
19	.41	.39	.29	.22	.17		.61	.63	.56	.52	.51
18	.44	.43	.32	.25	.21		.64	.66	.59	.55	.54
17	.47	.46	.35	.28	.24		.67	.69	.62	.58	.56
16	.50	.49	.39	.32	.28		.70	.72	.66	.62	.61
15	.54	.52	.42	.35	.31		.73	.75	.69	.65	.64
14	.57	.56	.45	.38	.34		.76	.79	.72	.68	.67
13	.60	.59	.49	.42	.38		.79	.82	.75	.71	.71
12	.63	.62	.52	.45	.41		.82	.85	.78	.75	.74
11	.67	.65	.55	.48	.45		.85	.88	.82	.78	.77
10	.70	.68	.59	.52	.49		.88	.91	.85	.81	.80
09	.73	.72	.62	.55	.51		.91	.94	.88	.84	.84
08	.76	.75	.65	.59	.55		.94	.97	.91	.87	.87
07	.79	.78	.69	.62	.58		.98	.04	.94	.91	.90
06	.83	.81	.72	.65	.62		90.01	.04	.97	.94	.93
05	.86	.85	.75	.69	.65		.04	.07	88.01	.97	.97
04	.89	.88	.78	.72	.68		.07	.10	.04	87.00	86.00
03	.92	.91	.82	.75	.72		.10	.13	.07	.04	.03
02	.95	.94	.85	.79	.75		.13	.16	.10	.07	.06
01	.99	.98	.89	.82	.79		.16	.19	.13	.10	.10
00	88.02	87.01	.92	.85	.82		.19	.22	.16	.13	.13
0.8399	.05	.04	.95	.89	.85		.22	.25	.19	.16	.16
98	.08	.07	.98	.92	.89		.25	.28	.21	.19	.19
97	.11	.10	86.02	.95	.92		.28	.31	.26	.23	.23
96	.14	.14	.05	.99	.96		.31	.35	.29	.26	.26
95	.18	.17	.08	85.02	.99		.34	.38	.32	.29	.29
94	.21	.20	.11	.05	84.02		.37	.41	.35	.32	.32
93	.24	.23	.15	.09	.06		.40	.44	.38	.35	.35
92	.27	.27	.18	.12	.09		.43	.47	.41	.39	.39
91	.30	.30	.21	.15	.12		.46	.50	.45	.42	.42
90	.33	.33	.24	.18	.16		.49	.53	.48	.45	.45

REFERENCE TABLES

XLII

Percentages by volume at 60°F of ethyl alcohol corresponding to apparent specific gravities at various temperatures.—Continued.

APPARENT SPECIFIC GRAVITY	15.56						APPARENT SPECIFIC GRAVITY	15.56					
	15.56	20/20	25/25	30/30	35/35			15.56	20/20	25/25	30/30	35/35	
0.8320	90.49	89.53	88.48	87.45	86.45		0.8250	92.53	91.62	90.61	89.64	88.67	
19	.51	.56	.51	.48	.48		49	.35	.64	.64	.67	.70	
18	.54	.59	.54	.51	.52		48	.38	.67	.67	.70	.73	
17	.57	.62	.57	.54	.55		47	.61	.70	.70	.73	.76	
16	.60	.65	.60	.58	.58		46	.64	.73	.73	.76	.79	
15	.63	.68	.64	.61	.61		45	.66	.76	.76	.79	.83	
14	.66	.71	.67	.64	.65		44	.69	.79	.79	.82	.86	
13	.69	.74	.70	.67	.68		43	.72	.82	.82	.85	.89	
12	.72	.77	.73	.70	.71		42	.75	.85	.85	.88	.92	
11	.75	.80	.76	.74	.74		41	.78	.87	.88	.91	.95	
10	.78	.83	.79	.77	.77		40	.80	.90	.91	.94	.98	
09	.81	.86	.82	.80	.81		39	.83	.93	.94	.97	99.01	
08	.84	.89	.85	.83	.84		38	.86	.96	.97	99.00	.04	
07	.87	.93	.88	.86	.87		37	.89	.99	91.00	.03	.07	
06	.90	.96	.92	.89	.90		36	.92	.92	.93	.96	.10	
05	.93	.99	.95	.93	.94		35	.94	.95	.96	.99	.13	
04	.96	90.02	.98	.96	.97		34	.97	.98	.99	.12	.16	
03	.99	.05	89.01	.99	87.00		33	93.00	.10	.12	.15	.20	
02	91.02	.08	.04	88.02	.03		32	.03	.13	.15	.18	.23	
01	.06	.11	.07	.05	.06		31	.05	.16	.18	.21	.26	
00	.08	.14	.10	.08	.10		30	.08	.19	.21	.24	.29	
.08299	.11	.17	.13	.12	.13		29	.11	.22	.23	.27	.32	
98	.14	.20	.16	.15	.16		28	.14	.25	.26	.30	.35	
97	.17	.23	.19	.18	.19		27	.16	.28	.29	.33	.38	
96	.20	.26	.22	.21	.22		26	.19	.31	.32	.36	.41	
95	.23	.29	.25	.24	.25		25	.22	.33	.35	.39	.44	
94	.26	.32	.28	.27	.29		24	.25	.36	.38	.42	.47	
93	.28	.35	.31	.30	.32		23	.27	.39	.41	.45	.50	
92	.31	.38	.35	.34	.35		22	.30	.42	.44	.48	.53	
91	.34	.41	.38	.37	.38		21	.33	.45	.47	.51	.56	
90	.37	.44	.41	.40	.41		20	.36	.48	.50	.54	.59	
89	.40	.47	.44	.43	.44		19	.38	.50	.52	.57	.62	
88	.43	.50	.47	.46	.48		18	.41	.53	.55	.60	.65	
87	.46	.53	.50	.49	.51		17	.44	.56	.58	.63	.68	
86	.49	.56	.53	.52	.54		16	.47	.59	.61	.66	.71	
85	.52	.59	.56	.55	.57		15	.49	.62	.64	.69	.74	
84	.55	.62	.59	.59	.60		14	.52	.65	.67	.72	.77	
83	.58	.65	.62	.62	.63		13	.55	.67	.70	.75	.80	
82	.60	.67	.65	.65	.67		12	.58	.70	.73	.78	.84	
81	.63	.70	.68	.68	.70		11	.60	.73	.76	.81	.87	
80	.66	.73	.71	.71	.73		10	.63	.76	.79	.84	.90	
79	.69	.76	.74	.74	.76		09	.66	.79	.81	.87	.93	
78	.72	.79	.77	.77	.79		08	.68	.81	.84	.90	.96	
77	.75	.82	.80	.81	.83		07	.71	.84	.87	.93	.99	
76	.78	.85	.83	.84	.86		06	.74	.87	.90	.96	99.02	
75	.81	.88	.87	.87	.89		05	.76	.90	.93	.99	.05	
74	.84	.91	.90	.90	.92		04	.79	.92	.96	91.01	.08	
73	.87	.94	.93	.93	.95		03	.82	.95	.99	.04	.11	
72	.90	.97	.96	.96	.98		02	.84	.98	92.02	.07	.14	
71	.92	91.00	.99	.99	88.02		01	.87	93.01	.05	.10	.17	
70	.95	.03	90.02	89.02	.05		00	.90	.04	.07	.13	.20	
69	.98	.06	.05	.06	.08		0.8199	.92	.08	.10	.16	.23	
68	92.01	.09	.08	.09	.11		98	.95	.09	.13	.19	.26	
67	.04	.12	.11	.12	.14		97	.98	.12	.16	.22	.29	
66	.07	.15	.14	.15	.17		96	94.01	.14	.19	.25	.32	
65	.10	.18	.17	.18	.20		95	.03	.17	.22	.27	.35	
64	.13	.21	.20	.21	.23		94	.06	.20	.24	.30	.38	
63	.15	.24	.23	.24	.26		93	.08	.23	.27	.33	.40	
62	.18	.27	.26	.27	.30		92	.11	.25	.30	.36	.43	
61	.21	.29	.29	.30	.33		91	.14	.28	.33	.39	.46	
60	.24	.32	.32	.33	.36		90	.16	.31	.35	.42	.49	
59	.27	.35	.35	.36	.39		89	.19	.34	.39	.45	.52	
58	.30	.38	.38	.39	.42		88	.22	.36	.41	.48	.55	
57	.33	.41	.41	.42	.45		87	.24	.39	.44	.51	.58	
56	.35	.44	.44	.45	.48		86	.27	.42	.47	.53	.61	
55	.38	.47	.47	.48	.51		85	.30	.44	.50	.56	.64	
54	.41	.50	.50	.51	.55		84	.32	.47	.53	.59	.67	
53	.44	.53	.53	.54	.58		83	.35	.50	.56	.62	.70	
52	.47	.56	.56	.57	.61		82	.38	.53	.58	.65	.73	
51	.50	.59	.59	.61	.64		81	.40	.55	.61	.68	.76	
50	.53	.62	.61	.64	.67		80	.43	.58	.64	.71	.79	

19 Percentages by volume at 60°F of ethyl alcohol corresponding to apparent specific gravities at various temperatures.—Continued.

APPARENT SPECIFIC GRAVITY	15.56	20/20	25/25	30/30	35/35	APPARENT SPECIFIC GRAVITY	15.56	20/20	25/25	30/30	35/35
	15.56	20/20	25/25	30/30	35/35		15.56	20/20	25/25	30/30	35/35
0.8180	94.43	93.58	92.64	91.71	90.79	0.8110	96.20	95.42	94.53	93.67	92.80
79	.46	.61	.67	.74	.82	09	.23	.44	.56	.69	.83
78	.48	.64	.70	.77	.85	08	.25	.47	.59	.72	.86
77	.51	.66	.72	.79	.88	07	.28	.49	.61	.75	.89
76	.53	.69	.75	.82	.91	06	.30	.52	.64	.77	.92
75	.56	.72	.78	.85	.94	05	.32	.54	.66	.80	.94
74	.59	.74	.81	.88	.97	04	.35	.57	.69	.83	.97
73	.61	.77	.84	.91	91.00	03	.37	.59	.72	.85	93.00
72	.64	.80	.86	.94	.03	02	.40	.62	.74	.88	.03
71	.66	.82	.89	.97	.06	01	.42	.64	.77	.91	.05
70	.69	.85	.92	92.00	.09	00	.45	.67	.79	.94	.08
69	.72	.88	.95	.03	.12	0.8099	.47	.69	.82	.96	.11
68	.74	.90	.97	.05	.14	98	.50	.72	.85	.99	.14
67	.77	.93	93.00	.08	.17	97	.52	.74	.87	94.02	.16
66	.79	.96	.03	.11	.20	96	.54	.77	.90	.04	.19
65	.82	.98	.06	.14	.23	95	.57	.79	.92	.07	.22
64	.84	94.01	.09	.17	.26	94	.59	.82	.95	.10	.25
63	.87	.04	.11	.20	.29	93	.61	.84	.98	.12	.27
62	.90	.06	.14	.22	.32	92	.64	.87	95.00	.15	.30
61	.92	.09	.17	.25	.35	91	.66	.89	.03	.17	.33
60	.95	.12	.20	.28	.38	90	.69	.92	.05	.20	.36
59	.97	.14	.22	.31	.40	89	.71	.94	.08	.23	.38
58	95.00	.17	.25	.34	.43	88	.73	.97	.10	.25	.41
57	.03	.20	.28	.36	.46	87	.75	.99	.13	.28	.44
56	.05	.22	.30	.39	.49	86	.78	96.02	.16	.31	.46
55	.08	.25	.33	.42	.52	85	.81	.04	.18	.33	.49
54	.10	.28	.36	.45	.55	84	.83	.07	.21	.36	.52
53	.13	.30	.39	.48	.58	83	.85	.09	.23	.39	.55
52	.15	.33	.41	.51	.61	82	.88	.11	.26	.41	.57
51	.18	.36	.44	.54	.64	81	.90	.14	.28	.44	.60
50	.20	.38	.47	.56	.66	80	.93	.16	.31	.47	.63
49	.23	.41	.50	.59	.69	79	.95	.19	.35	.49	.65
48	.25	.44	.52	.62	.72	78	.97	.21	.36	.52	.68
47	.28	.46	.55	.65	.75	77	97.00	.24	.39	.54	.71
46	.30	.49	.58	.68	.78	76	.02	.26	.41	.57	.73
45	.33	.51	.60	.70	.81	75	.04	.29	.44	.60	.76
44	.36	.54	.63	.73	.84	74	.07	.31	.46	.62	.79
43	.38	.57	.66	.76	.87	73	.09	.33	.49	.65	.81
42	.41	.59	.69	.79	.90	72	.11	.36	.51	.67	.84
41	.43	.62	.71	.82	.92	71	.14	.38	.54	.70	.87
40	.46	.64	.74	.84	.95	70	.16	.41	.56	.73	.90
39	.48	.67	.77	.87	.98	69	.18	.43	.59	.75	.92
38	.51	.70	.79	.90	92.01	68	.21	.46	.61	.78	.95
37	.53	.72	.82	.93	.04	67	.23	.48	.64	.80	.98
36	.56	.75	.85	.96	.07	66	.25	.50	.66	.83	94.00
35	.58	.77	.87	.98	.10	65	.28	.53	.69	.86	.03
34	.61	.80	.90	93.01	.13	64	.30	.55	.71	.88	.06
33	.63	.83	.93	.04	.15	63	.32	.58	.74	.91	.08
32	.66	.85	.95	.07	.18	62	.35	.60	.76	.93	.11
31	.68	.88	.98	.09	.21	61	.37	.63	.79	.96	.14
30	.71	.90	94.01	.12	.24	60	.39	.65	.81	.99	.16
29	.73	.93	.03	.15	.27	59	.42	.67	.84	95.01	.19
28	.76	.95	.06	.18	.30	58	.44	.70	.86	.04	.22
27	.78	.98	.09	.20	.33	57	.46	.72	.89	.06	.24
26	.81	95.01	.11	.23	.35	56	.49	.75	.91	.09	.27
25	.83	.03	.14	.26	.38	55	.51	.77	.94	.11	.30
24	.86	.06	.17	.29	.41	54	.53	.79	.96	.14	.32
23	.88	.08	.19	.31	.44	53	.55	.82	.99	.17	.35
22	.91	.11	.22	.34	.47	52	.58	.84	96.01	.19	.38
21	.93	.14	.24	.37	.50	51	.60	.86	.04	.22	.40
20	.96	.16	.27	.40	.53	50	.62	.89	.06	.24	.43
19	.98	.19	.30	.42	.55	49	.64	.91	.09	.27	.45
18	99.01	.21	.32	.45	.58	48	.67	.94	.11	.29	.48
17	.03	.24	.35	.48	.61	47	.69	.96	.14	.32	.51
16	.06	.26	.38	.51	.64	46	.71	.98	.16	.34	.53
15	.08	.29	.40	.53	.66	45	.73	97.01	.19	.37	.56
14	.10	.32	.43	.56	.69	44	.76	.03	.21	.40	.59
13	.13	.34	.46	.59	.72	43	.78	.05	.24	.42	.61
12	.15	.37	.48	.61	.75	42	.80	.08	.26	.45	.64
11	.18	.39	.51	.64	.78	41	.82	.10	.28	.47	.66
10	.20	.42	.53	.67	.80	40	.85	.12	.31	.50	.69

Percentages by volume at 60°F of ethyl alcohol corresponding to apparent specific gravities at various temperatures.—Continued.

APPARENT SPECIFIC GRAVITY	15.56 15.56	20/20	25/25	30/30	35/35	APPARENT SPECIFIC GRAVITY	15.56 15.56	20/20	25/25	30/30	35/35
0.8040	97.85	97.12	96.31	95.50	94.69	0.7970	99.33	98.68	97.95	97.21	96.46
39	.87	.15	.33	.52	.72	69	.35	.70	.97	.23	.48
38	.89	.17	.36	.55	.74	68	.37	.72	.99	.25	.51
37	.91	.19	.38	.57	.77	67	.39	.75	98.02	.28	.53
36	.94	.22	.41	.60	.79	66	.42	.77	.04	.30	.56
35	.96	.24	.43	.62	.82	65	.44	.79	.06	.32	.58
34	.98	.26	.46	.65	.85	64	.46	.81	.08	.35	.60
33	98.00	.29	.48	.67	.87	63	.48	.83	.10	.37	.63
32	.03	.31	.50	.70	.90	62	.50	.85	.12	.39	.65
31	.05	.33	.53	.72	.92	61	.52	.87	.15	.42	.68
30	.07	.36	.55	.75	.95	60	.54	.89	.17	.44	.70
29	.09	.38	.58	.77	.98	59	.56	.91	.19	.46	.72
28	.11	.40	.60	.80	95.00	58	.58	.93	.21	.49	.75
27	.14	.43	.62	.82	.03	57	.60	.95	.23	.51	.77
26	.16	.45	.65	.85	.05	56	.61	.97	.26	.53	.80
25	.18	.47	.67	.87	.08	55	.63	99.00	.28	.56	.82
24	.20	.49	.70	.90	.11	54	.65	.02	.30	.58	.84
23	.22	.52	.72	.92	.13	53	.67	.04	.32	.60	.87
22	.25	.54	.74	.95	.16	52	.69	.06	.34	.62	.89
21	.27	.56	.77	.97	.18	51	.71	.08	.36	.65	.92
20	.29	.59	.79	96.00	.21	50	.73	.10	.39	.67	.94
19	.31	.61	.82	.02	.23	49	.75	.12	.41	.69	.96
18	.33	.63	.84	.05	.26	48	.77	.14	.43	.71	.99
17	.35	.66	.86	.07	.28	47	.79	.16	.45	.74	97.01
16	.38	.68	.89	.10	.31	46	.81	.18	.47	.76	.04
15	.40	.70	.91	.12	.34	45	.83	.20	.49	.78	.06
14	.42	.72	.94	.15	.36	44	.85	.22	.51	.80	.08
13	.44	.75	.96	.17	.39	43	.87	.24	.54	.83	.11
12	.46	.77	.98	.20	.41	42	.89	.26	.56	.85	.13
11	.48	.79	97.01	.22	.44	41	.91	.28	.58	.87	.15
10	.50	.81	.03	.25	.46	40	.93	.30	.60	.89	.18
09	.53	.84	.05	.27	.49	39	.95	.32	.62	.92	.20
08	.55	.86	.08	.29	.52	38	.97	.34	.64	.94	.22
07	.57	.88	.10	.32	.54	37	.99	.36	.66	.96	.25
06	.58	.90	.12	.34	.57	36	.00.00	.38	.68	.98	.27
05	.61	.92	.15	.37	.59	35	.40	.70	98.01	.29	.29
04	.63	.95	.17	.39	.62	34	.42	.73	.03	.32	.32
03	.65	.97	.19	.42	.64	33	.44	.75	.05	.34	.34
02	.67	.99	.22	.44	.67	32	.46	.77	.07	.36	.36
01	.70	98.01	.24	.47	.69	31	.48	.79	.09	.39	.39
00	.72	.03	.26	.49	.72	30	.50	.81	.12	.41	.41
0.7999	.74	.06	.29	.51	.74	29	.52	.83	.14	.43	.43
98	.76	.08	.31	.54	.77	28	.54	.85	.16	.46	.46
97	.78	.10	.33	.56	.79	27	.56	.87	.18	.48	.48
96	.80	.12	.36	.59	.82	26	.58	.89	.20	.50	.50
95	.82	.14	.38	.61	.84	25	.60	.91	.23	.52	.52
94	.84	.17	.40	.63	.87	24	.62	.93	.25	.55	.55
93	.86	.19	.43	.66	.89	23	.64	.96	.27	.57	.57
92	.88	.21	.45	.68	.92	22	.66	.98	.29	.59	.59
91	.90	.23	.47	.71	.94	21	.68	99.00	.31	.62	.62
90	.92	.26	.50	.73	.97	20	.70	.02	.33	.64	.64
89	.95	.28	.52	.75	.99	19	.72	.04	.36	.66	.66
88	.97	.30	.54	.78	96.02	18	.74	.06	.38	.68	.68
87	.99	.32	.57	.80	.04	17	.76	.08	.40	.71	.71
86	99.01	.34	.59	.83	.07	16	.78	.10	.42	.73	.73
85	.03	.36	.61	.85	.09	15	.80	.12	.44	.75	.75
84	.05	.39	.63	.87	.12	14	.82	.14	.46	.77	.77
83	.07	.41	.66	.90	.14	13	.84	.16	.48	.80	.80
82	.09	.43	.68	.92	.16	12	.86	.18	.51	.82	.82
81	.11	.45	.70	.95	.19	11	.88	.20	.53	.84	.84
80	.13	.47	.72	.97	.21	10	.90	.22	.55	.86	.86
79	.15	.49	.75	97.00	.24	09	.92	.24	.57	.89	.89
78	.17	.51	.77	.02	.26	08	.94	.27	.59	.91	.91
77	.19	.54	.79	.04	.29	07	.96	.29	.61	.93	.93
76	.21	.56	.81	.07	.31	06	.98	.31	.63	.95	.95
75	.23	.58	.84	.09	.34	05	100.00	.33	.66	.98	.98
74	.25	.60	.86	.11	.36	04	.35	.68	98.00	.00	.00
73	.27	.62	.88	.14	.38	03	.37	.70	.02	.02	.02
72	.29	.64	.90	.16	.41	02	.39	.72	.04	.04	.04
71	.31	.66	.93	.18	.43	01	.41	.74	.07	.07	.07
70	.33	.68	.95	.21	.46	00	.43	.76	.09	.09	.09



19 Percentages by volume at 60°F of ethyl alcohol corresponding to apparent specific gravities at various temperatures.—Concluded.

APPARENT SPECIFIC GRAVITY	25/25	30/30	35/35	APPARENT SPECIFIC GRAVITY	35/35
0.7900	99.43	98.76	98.09	0.7830	99.56
.7899	.45	.78	.11		.58
.98	.47	.80	.13		.60
.97	.49	.82	.15		.62
.96	.51	.84	.18		.64
.95	.53	.87	.20		.66
.94	.55	.89	.22		.68
.93	.57	.91	.24		.70
.92	.59	.93	.26		.72
.91	.61	.95	.29		.74
.90	.63	.97	.31		.76
.89	.65	.99	.33		.78
.88	.67	99.01	.35		.80
.87	.69	.03	.37		.82
.86	.71	.05	.40		.84
.85	.73	.07	.42		.86
.84	.75	.09	.44		.88
.83	.77	.12	.46		.90
.82	.79	.14	.48		.92
.81	.81	.16	.50		.94
.80	.83	.18	.53	.09	.98
.79	.85	.20	.55	.08	100.00
.78	.86	.22	.57		
.77	.88	.24	.59		
.76	.90	.26	.61		
.75	.92	.28	.63		
.74	.94	.30	.65		
.73	.96	.32	.67		
.72	.98	.34	.70		
.71	100.00	.36	.72		
.70		.38	.74		
.69		.40	.76		
.68		.42	.78		
.67		.44	.80		
.66		.46	.82		
.65		.48	.84		
.64		.50	.86		
.63		.52	.88		
.62		.54	.91		
.61		.56	.93		
.60		.58	.95		
.59		.60	.97		
.58		.62	.99		
.57		.64	99.01		
.56		.66	.03		
.55		.68	.05		
.54		.70	.07		
.53		.72	.09		
.52		.74	.12		
.51		.76	.14		
.50		.78	.16		
.49		.80	.18		
.48		.72	.20		
.47		.84	.22		
.46		.86	.24		
.45		.88	.26		
.44		.90	.28		
.43		.92	.30		
.42		.94	.32		
.41		.96	.34		
.40		.98	.36		
.39		100.00	.38		
.38			.40		
.37			.42		
.36			.44		
.35			.46		
.34			.48		
.33			.50		
.32			.52		
.31			.54		
.30			.56		

Alcohol table for calculating the percentages of alcohol by volume at 15.56°C in 20 mixtures of ethyl alcohol and water from their Zeiss immersion refractometer readings and indices of refraction at 17.5–25°C.<sup>1</sup>

SCALE READING <sup>2</sup>	INDEX OF REFRACTION	17.5° C.	18° C.	19° C.	20° C.	21° C.	22° C.	23° C.	24° C.	25° C.
		Per cent by volume	Per cent by volume	Per cent by volume	Per cent by volume	Per cent by volume	Per cent by volume	Per cent by volume	Per cent by volume	Per cent by volume
13.2	1.33250	....	....	....	....	....	....	....	....	0.00
13.4	3257	....	....	....	....	....	....	....	....	0.18
13.6	3265	....	....	....	....	....	....	....	0.14	0.35
13.8	3273	....	....	....	....	....	....	0.10	0.31	0.53
14.0	3281	....	....	....	....	0.08	0.28	0.49	0.70	
14.2	3288	....	....	....	0.04	0.24	0.45	0.67	0.88	
14.4	3296	....	....	....	0.21	0.41	0.63	0.84	1.06	
14.6	3304	....	....	0.16	0.38	0.59	0.80	1.02	1.24	
14.8	3312	....	....	0.14	0.34	0.55	0.77	0.98	1.19	1.40
15.0	3319	0.00	0.10	0.31	0.52	0.73	0.94	1.16	1.36	1.55
15.2	3327	0.17	0.27	0.48	0.69	0.91	1.12	1.32	1.51	1.71
15.4	3335	0.34	0.44	0.65	0.85	1.07	1.29	1.47	1.66	1.86
15.6	3343	0.51	0.60	0.82	1.03	1.24	1.44	1.62	1.82	2.01
15.8	3350	0.68	0.78	0.99	1.21	1.40	1.60	1.77	1.97	2.17
16.0	3358	0.84	0.94	1.17	1.36	1.55	1.75	1.92	2.12	2.33
16.2	3366	1.02	1.12	1.32	1.51	1.70	1.90	2.08	2.27	2.48
16.4	3374	1.18	1.29	1.47	1.66	1.85	2.05	2.24	2.43	2.62
16.6	3381	1.34	1.43	1.62	1.81	2.00	2.20	2.39	2.57	2.77
16.8	3389	1.49	1.57	1.77	1.96	2.15	2.35	2.53	2.72	2.92
17.0	3397	1.63	1.72	1.92	2.11	2.30	2.50	2.69	2.87	3.06
17.2	3405	1.77	1.87	2.06	2.26	2.45	2.65	2.82	3.02	3.21
17.4	3412	1.92	2.01	2.21	2.41	2.59	2.79	2.97	3.17	3.36
17.6	3420	2.07	2.16	2.36	2.56	2.74	2.94	3.12	3.32	3.51
17.8	3428	2.21	2.31	2.51	2.70	2.89	3.09	3.27	3.46	3.66
18.0	3435	2.36	2.45	2.66	2.85	3.04	3.23	3.42	3.61	3.81
18.2	3443	2.50	2.60	2.81	3.00	3.19	3.37	3.57	3.76	3.96
18.4	3451	2.65	2.75	2.96	3.15	3.34	3.52	3.71	3.91	4.11
18.6	3459	2.80	2.90	3.10	3.30	3.48	3.66	3.86	4.06	4.26
18.8	3466	2.95	3.05	3.25	3.45	3.63	3.81	4.01	4.21	4.41
19.0	3474	3.10	3.19	3.40	3.59	3.77	3.96	4.16	4.36	4.56
19.2	3482	3.25	3.34	3.55	3.73	3.92	4.11	4.31	4.51	4.70
19.4	3489	3.39	3.48	3.70	3.88	4.07	4.26	4.46	4.65	4.85
19.6	3497	3.53	3.63	3.84	4.03	4.22	4.41	4.61	4.80	5.00
19.8	3505	3.68	3.78	3.98	4.17	4.37	4.56	4.75	4.95	5.15
20.0	3513	3.83	3.93	4.13	4.32	4.52	4.72	4.90	5.10	5.29
20.2	3520	3.97	4.07	4.27	4.47	4.66	4.87	5.05	5.24	5.44
20.4	3528	4.12	4.22	4.42	4.61	4.82	5.01	5.20	5.38	5.58
20.6	3536	4.26	4.36	4.56	4.75	4.96	5.15	5.34	5.52	5.72
20.8	3543	4.41	4.51	4.70	4.90	5.10	5.29	5.48	5.67	5.87
21.0	3551	4.56	4.65	4.85	5.04	5.24	5.44	5.62	5.82	6.02
21.2	3559	4.70	4.80	4.99	5.19	5.39	5.58	5.77	5.96	6.16
21.4	3566	4.84	4.94	5.14	5.33	5.53	5.72	5.91	6.11	6.30
21.6	3574	4.99	5.09	5.28	5.47	5.67	5.87	6.06	6.25	6.44
21.8	3582	5.13	5.23	5.43	5.61	5.82	6.01	6.20	6.39	6.59

<sup>1</sup> Rearranged from the table of B. H. St. John, which is based upon the data of Doroshevskii and Dvorschanik, *J. Russ. Phys. Chem. Soc.*, 40, 101 (1908). The scale readings were converted into refractive indices by using the formula  $n_D = 1.327338 + 0.00039347X + 0.0000002046X^2$ .

<sup>2</sup> The scale readings refer only to the scale of arbitrary units proposed by Fulrich, *Z. anorg. Chem.*, p. 1168, 1899. According to this scale, 14.5 = 1.33300, 50.0 = 1.34650, and 100.0 = 1.36464. If the immersion refractometer used is calibrated to another arbitrary scale, the readings must be converted into refractive indices before the table is used to determine the percentage of alcohol.

SCALE READING	INDEX OF REFRACTION	17.5° C.	18° C.	19° C.	20° C.	21° C.	22° C.	23° C.	24° C.	25° C.
		Per cent by volume	Per cent by volume	Per cent by volume	Per cent by volume	Per cent by volume	Per cent by volume	Per cent by volume	Per cent by volume	Per cent by volume
22.0	33590	5.27	5.37	5.57	5.76	5.96	6.15	6.34	6.54	6.73
22.2	3597	5.41	5.51	5.71	5.90	6.11	6.29	6.49	6.68	6.87
22.4	3605	5.56	5.65	5.85	6.05	6.25	6.43	6.63	6.82	7.01
22.6	3613	5.70	5.80	6.00	6.19	6.39	6.57	6.77	6.96	7.16
22.8	3620	5.85	5.94	6.14	6.33	6.53	6.71	6.91	7.10	7.31
23.0	3628	5.99	6.08	6.28	6.47	6.67	6.86	7.06	7.24	7.45
23.2	3636	6.13	6.22	6.42	6.61	6.81	7.00	7.20	7.39	7.59
23.4	3643	6.27	6.36	6.56	6.75	6.95	7.14	7.34	7.53	7.73
23.6	3651	6.41	6.50	6.70	6.90	7.09	7.28	7.48	7.67	7.87
23.8	3659	6.55	6.64	6.85	7.04	7.23	7.42	7.62	7.81	8.00
24.0	3666	6.69	6.78	6.99	7.18	7.38	7.56	7.76	7.95	8.14
24.2	3674	6.83	6.92	7.13	7.32	7.52	7.70	7.90	8.09	8.28
24.4	3682	6.97	7.06	7.27	7.46	7.66	7.84	8.04	8.23	8.42
24.6	3689	7.11	7.20	7.41	7.60	7.80	7.98	8.17	8.37	8.55
24.8	3697	7.25	7.35	7.55	7.74	7.93	8.12	8.31	8.51	8.69
25.0	3705	7.39	7.49	7.68	7.88	8.06	8.26	8.45	8.64	8.84
25.2	3712	7.53	7.63	7.82	8.01	8.20	8.40	8.59	8.78	8.98
25.4	3720	7.66	7.76	7.95	8.14	8.34	8.54	8.73	8.92	9.12
25.6	3728	7.80	7.90	8.09	8.28	8.48	8.68	8.86	9.06	9.26
25.8	3735	7.94	8.03	8.22	8.42	8.62	8.82	9.00	9.20	9.39
26.0	3743	8.07	8.16	8.36	8.55	8.75	8.95	9.14	9.34	9.53
26.2	3751	8.21	8.30	8.50	8.69	8.89	9.09	9.28	9.48	9.67
26.4	3758	8.34	8.44	8.63	8.82	9.03	9.22	9.42	9.61	9.81
26.6	3766	8.48	8.57	8.77	8.96	9.16	9.36	9.55	9.75	9.95
26.8	3774	8.62	8.71	8.91	9.10	9.30	9.49	9.69	9.89	10.09
27.0	3781	8.75	8.85	9.05	9.23	9.44	9.63	9.83	10.03	10.23
27.2	3789	8.89	8.98	9.18	9.37	9.58	9.76	9.97	10.17	10.37
27.4	3796	9.02	9.12	9.32	9.51	9.71	9.90	10.10	10.31	10.51
27.6	3804	9.16	9.26	9.45	9.65	9.85	10.03	10.24	10.45	10.65
27.8	3812	9.29	9.39	9.59	9.79	9.98	10.17	10.38	10.58	10.79
28.0	3820	9.43	9.53	9.72	9.92	10.12	10.31	10.51	10.72	10.93
28.2	3827	9.57	9.66	9.86	10.06	10.25	10.45	10.65	10.86	11.06
28.4	3835	9.70	9.80	9.99	10.19	10.39	10.59	10.79	11.00	11.20
28.6	3842	9.84	9.93	10.13	10.32	10.52	10.72	10.93	11.13	11.33
28.8	3850	9.97	10.07	10.26	10.46	10.66	10.86	11.06	11.27	11.47
29.0	3858	10.10	10.19	10.40	10.59	10.79	11.00	11.20	11.40	11.61
29.2	3865	10.24	10.33	10.52	10.73	10.93	11.13	11.33	11.54	11.75
29.4	3873	10.36	10.46	10.66	10.86	11.06	11.27	11.47	11.67	11.88
29.6	3881	10.50	10.59	10.79	10.99	11.20	11.39	11.60	11.81	12.01
29.8	3888	10.63	10.72	10.93	11.12	11.33	11.53	11.74	11.94	12.15
30.0	3896	10.76	10.86	11.05	11.26	11.46	11.66	11.87	12.08	12.29
30.2	3904	10.89	10.99	11.18	11.38	11.59	11.79	12.00	12.21	12.42
30.4	3911	11.02	11.12	11.31	11.51	11.72	11.93	12.13	12.34	12.56
30.6	3919	11.15	11.25	11.44	11.64	11.85	12.06	12.27	12.48	12.70
30.8	3926	11.28	11.38	11.58	11.78	11.99	12.19	12.40	12.61	12.84

Alcohol table.—Continued.

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SCALE READING	INDEX OF REFRACTION	17.5° C.	18° C.	19° C.	20° C.	21° C.	22° C.	23° C.	24° C.	25° C.
		Per cent by volume	Per cent by volume	Per cent by volume	Per cent by volume	Per cent by volume	Per cent by volume	Per cent by volume	Per cent by volume	Per cent by volume
31.0	1.33934	11.41	11.51	11.71	11.91	12.12	12.32	12.54	12.75	12.97
31.2	3942	11.54	11.64	11.84	12.04	12.25	12.46	12.67	12.89	13.11
31.4	3949	11.66	11.77	11.97	12.17	12.38	12.59	12.81	13.02	13.24
31.6	3957	11.79	11.90	12.10	12.30	12.51	12.72	12.94	13.15	13.37
31.8	3964	11.92	12.03	12.23	12.43	12.64	12.85	13.07	13.29	13.51
32.0	3972	12.05	12.15	12.36	12.57	12.78	12.99	13.20	13.42	13.64
32.2	3980	12.18	12.28	12.49	12.70	12.91	13.12	13.34	13.55	13.77
32.4	3987	12.31	12.40	12.62	12.83	13.04	13.25	13.47	13.69	13.91
32.6	3995	12.43	12.54	12.75	12.96	13.17	13.38	13.60	13.82	14.04
32.8	4002	12.56	12.67	12.88	13.09	13.30	13.51	13.73	13.95	14.17
33.0	4010	12.69	12.79	13.01	13.22	13.43	13.64	13.86	14.09	14.31
33.2	4018	12.82	12.92	13.13	13.35	13.56	13.78	13.99	14.22	14.44
33.4	4025	12.95	13.05	13.26	13.48	13.69	13.91	14.13	14.35	14.58
33.6	4033	13.08	13.18	13.39	13.61	13.82	14.04	14.26	14.48	14.71
33.8	4040	13.20	13.30	13.52	13.74	13.95	14.17	14.39	14.62	14.85
34.0	4048	13.33	13.43	13.64	13.86	14.08	14.30	14.52	14.75	14.98
34.2	4056	13.45	13.56	13.77	13.99	14.21	14.43	14.65	14.88	15.11
34.4	4063	13.58	13.68	13.90	14.12	14.34	14.57	14.78	15.01	15.25
34.6	4071	13.70	13.81	14.02	14.25	14.47	14.70	14.91	15.14	15.38
34.8	4078	13.83	13.94	14.14	14.37	14.59	14.83	15.05	15.28	15.51
35.0	4086	13.96	14.06	14.27	14.50	14.72	14.96	15.18	15.41	15.65
35.2	4094	14.08	14.19	14.39	14.62	14.85	15.09	15.31	15.54	15.78
35.4	4101	14.21	14.31	14.52	14.75	14.97	15.22	15.44	15.67	15.91
35.6	4109	14.33	14.44	14.65	14.87	15.10	15.34	15.56	15.80	16.05
35.8	4116	14.46	14.56	14.78	15.00	15.23	15.47	15.69	15.93	16.18
36.0	4124	14.58	14.69	14.90	15.13	15.35	15.59	15.82	16.06	16.31
36.2	4131	14.71	14.81	15.03	15.25	15.48	15.72	15.95	16.19	16.44
36.4	4139	14.83	14.94	15.16	15.38	15.61	15.85	16.08	16.32	16.56
36.6	4146	14.96	15.06	15.28	15.51	15.73	15.97	16.21	16.45	16.69
36.8	4154	15.08	15.19	15.41	15.63	15.86	16.10	16.34	16.58	16.82
37.0	4162	15.20	15.31	15.53	15.76	15.99	16.23	16.47	16.71	16.95
37.2	4169	15.33	15.44	15.66	15.89	16.11	16.35	16.60	16.84	17.08
37.4	4177	15.45	15.56	15.79	16.01	16.24	16.48	16.72	16.97	17.21
37.6	4184	15.57	15.69	15.91	16.14	16.37	16.61	16.85	17.09	17.34
37.8	4192	15.70	15.81	16.04	16.26	16.49	16.73	16.98	17.22	17.46
38.0	4199	15.82	15.94	16.16	16.39	16.62	16.86	17.11	17.35	17.59
38.2	4207	15.94	16.06	16.29	16.51	16.75	16.99	17.23	17.47	17.72
38.4	4215	16.07	16.18	16.41	16.64	16.87	17.11	17.36	17.60	17.85
38.6	4222	16.19	16.31	16.53	16.76	17.00	17.24	17.48	17.73	17.97
38.8	4230	16.31	16.43	16.66	16.89	17.13	17.36	17.61	17.85	18.10
39.0	4237	16.44	16.55	16.78	17.01	17.25	17.49	17.74	17.98	18.23
39.2	4245	16.56	16.67	16.91	17.14	17.38	17.62	17.86	18.11	18.35
39.4	4252	16.68	16.80	17.03	17.26	17.50	17.74	17.99	18.23	18.48
39.6	4260	16.80	16.92	17.15	17.39	17.63	17.87	18.11	18.36	18.61
39.8	4267	16.93	17.04	17.28	17.51	17.75	17.99	18.24	18.48	18.73

SCALE READING	INDEX OF REFRACTION	17.5° C.	18° C.	18° C.	20° C.	21° C.	22° C.	23° C.	24° C.	25° C.
		Per cent by volume	Per cent by volume	Per cent by volume	Per cent by volume	Per cent by volume	Per cent by volume	Per cent by volume	Per cent by volume	Per cent by volume
40.0	1.34275	17.05	17.16	17.40	17.63	17.88	18.12	18.36	18.61	18.86
40.2	4282	17.17	17.29	17.52	17.76	18.00	18.24	18.49	18.74	18.99
40.4	4290	17.29	17.41	17.64	17.88	18.12	18.37	18.61	18.86	19.11
40.6	4298	17.41	17.53	17.77	18.01	18.25	18.49	18.74	18.99	19.24
40.8	4305	17.54	17.65	17.89	18.13	18.37	18.61	18.86	19.11	19.37
41.0	4313	17.66	17.77	18.01	18.25	18.49	18.74	18.99	19.24	19.49
41.2	4320	17.78	17.90	18.13	18.37	18.62	18.86	19.11	19.36	19.62
41.4	4328	17.90	18.03	18.26	18.50	18.74	18.99	19.24	19.49	19.75
41.6	4335	18.02	18.14	18.38	18.62	18.86	19.11	19.36	19.61	19.87
41.8	4343	18.14	18.26	18.50	18.74	18.99	19.23	19.48	19.74	20.00
42.0	4350	18.27	18.38	18.62	18.87	19.11	19.36	19.61	19.86	20.13
42.2	4358	18.39	18.50	18.74	18.99	19.23	19.48	19.73	19.99	20.25
42.4	4365	18.51	18.62	18.87	19.11	19.36	19.60	19.86	20.11	20.38
42.6	4373	18.63	18.75	18.99	19.23	19.48	19.72	19.98	20.24	20.50
42.8	4380	18.75	18.87	19.11	19.36	19.60	19.85	20.10	20.36	20.63
43.0	4388	18.87	18.99	19.23	19.48	19.72	19.97	20.23	20.49	20.75
43.2	4395	18.99	19.11	19.35	19.60	19.85	20.09	20.35	20.61	20.88
43.4	4403	19.11	19.23	19.47	19.72	19.97	20.21	20.47	20.74	21.01
43.6	4410	19.23	19.35	19.59	19.85	20.09	20.34	20.60	20.86	21.13
43.8	4418	19.35	19.47	19.72	19.97	20.21	20.46	20.72	20.99	21.25
44.0	4426	19.46	19.59	19.84	20.09	20.34	20.58	20.84	21.11	21.38
44.2	4433	19.58	19.71	19.96	20.21	20.46	20.71	20.96	21.23	21.50
44.4	4440	19.70	19.83	20.08	20.33	20.58	20.83	21.09	21.36	21.63
44.6	4448	19.82	19.95	20.20	20.45	20.70	20.95	21.21	21.48	21.75
44.8	4456	19.94	20.07	20.32	20.58	20.82	21.07	21.33	21.60	21.88
45.0	4463	20.06	20.18	20.44	20.70	20.95	21.19	21.45	21.73	22.00
45.2	4470	20.18	20.30	20.56	20.82	21.07	21.31	21.58	21.85	22.13
45.4	4478	20.29	20.42	20.68	20.94	21.19	21.43	21.70	21.98	22.25
45.6	4486	20.41	20.54	20.80	21.06	21.31	21.55	21.82	22.10	22.38
45.8	4493	20.53	20.66	20.92	21.18	21.43	21.67	21.94	22.23	22.51
46.0	4500	20.65	20.78	21.04	21.30	21.54	21.79	22.07	22.35	22.64
46.2	4508	20.76	20.89	21.16	21.42	21.66	21.91	22.19	22.48	22.76
46.4	4516	20.88	21.01	21.28	21.54	21.78	22.03	22.32	22.61	22.89
46.6	4523	21.00	21.13	21.40	21.66	21.90	22.16	22.44	22.73	23.02
46.8	4530	21.12	21.25	21.52	21.78	22.02	22.28	22.57	22.86	23.15
47.0	4538	21.24	21.37	21.64	21.90	22.15	22.41	22.69	22.99	23.28
47.2	4545	21.36	21.49	21.76	22.02	22.27	22.53	22.82	23.12	23.41
47.4	4553	21.48	21.61	21.88	22.15	22.39	22.66	22.94	23.24	23.54
47.6	4560	21.60	21.73	22.00	22.27	22.51	22.78	23.07	23.37	23.67
47.8	4568	21.72	21.85	22.12	22.39	22.64	22.91	23.20	23.50	23.80
48.0	4575	21.84	21.97	22.24	22.51	22.76	23.03	23.32	23.63	23.93
48.2	4583	21.96	22.09	22.36	22.63	22.88	23.16	23.45	23.76	24.06
48.4	4590	22.08	22.21	22.48	22.75	23.01	23.28	23.58	23.89	24.19
48.6	4598	22.20	22.33	22.60	22.87	23.13	23.41	23.71	24.02	24.32
48.8	4605	22.32	22.45	22.72	22.99	23.26	23.54	23.83	24.14	24.45

## Alcohol table.—Continued.

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SCALE READING	INDEX OF REFRACTION	17.5° C.	18° C.	19° C.	20° C.	21° C.	22° C.	23° C.	24° C.	25° C.
		Per cent by volume	Per cent by volume	Per cent by volume	Per cent by volume	Per cent by volume	Per cent by volume	Per cent by volume	Per cent by volume	Per cent by volume
49.0	1.34613	22.44	22.57	22.84	23.12	23.38	23.66	23.96	24.27	24.59
49.2	4620	22.56	22.69	22.96	23.24	23.51	23.79	24.09	24.40	24.72
49.4	4628	22.68	22.81	23.08	23.36	23.63	23.92	24.22	24.53	24.85
49.6	4635	22.80	22.93	23.21	23.48	23.76	24.04	24.35	24.66	24.98
49.8	4643	22.92	23.05	23.33	23.61	23.88	24.17	24.48	24.79	25.11
50.0	4650	23.04	23.17	23.45	23.73	24.01	24.30	24.61	24.92	25.25
50.2	4658	23.16	23.30	23.57	23.85	24.13	24.43	24.74	25.05	25.38
50.4	4665	23.28	23.42	23.69	23.98	24.26	24.56	24.86	25.18	25.51
50.6	4672	23.40	23.54	23.81	24.10	24.38	24.69	24.99	25.32	25.65
50.8	4680	23.51	23.66	23.93	24.22	24.51	24.81	25.12	25.45	25.78
51.0	4687	23.63	23.78	24.05	24.35	24.64	24.94	25.25	25.58	25.91
51.2	4695	23.75	23.90	24.18	24.47	24.76	25.07	25.38	25.71	26.05
51.4	4702	23.87	24.02	24.30	24.59	24.89	25.20	25.51	25.84	26.18
51.6	4710	23.99	24.14	24.42	24.72	25.01	25.33	25.64	25.97	26.32
51.8	4717	24.11	24.26	24.54	24.84	25.14	25.46	25.77	26.11	26.45
52.0	4724	24.23	24.38	24.66	24.96	25.27	25.58	25.90	26.24	26.59
52.2	4732	24.36	24.50	24.79	25.09	25.39	25.71	26.03	26.37	26.72
52.4	4740	24.48	24.62	24.91	25.21	25.52	25.84	26.16	26.51	26.86
52.6	4747	24.60	24.74	25.03	25.34	25.65	25.97	26.29	26.64	26.99
52.8	4754	24.72	24.86	25.15	25.46	25.77	26.10	26.42	26.77	27.13
53.0	4762	24.84	24.98	25.28	25.59	25.90	26.23	26.56	26.91	27.27
53.2	4769	24.96	25.10	25.40	25.71	26.03	26.35	26.69	27.04	27.40
53.4	4777	25.08	25.23	25.52	25.84	26.15	26.48	26.82	27.17	27.54
53.6	4784	25.20	25.35	25.65	25.96	26.28	26.61	26.95	27.31	27.67
53.8	4792	25.32	25.47	25.77	26.09	26.41	26.74	27.08	27.44	27.81
54.0	4799	25.44	25.59	25.90	26.22	26.54	26.87	27.21	27.58	27.95
54.2	4806	25.56	25.71	26.02	26.34	26.67	27.00	27.35	27.71	28.08
54.4	4814	25.68	25.84	26.14	26.47	26.79	27.13	27.48	27.85	28.22
54.6	4821	25.81	25.96	26.27	26.59	26.92	27.26	27.61	27.98	28.36
54.8	4829	25.93	26.08	26.39	26.72	27.05	27.39	27.75	28.11	28.49
55.0	4836	26.05	26.20	26.52	26.85	27.18	27.52	27.88	28.25	28.63
55.2	4844	26.17	26.32	26.64	26.97	27.31	27.65	28.01	28.38	28.77
55.4	4851	26.29	26.45	26.76	27.10	27.43	27.78	28.15	28.52	28.90
55.6	4858	26.41	26.57	26.89	27.23	27.55	27.92	28.28	28.65	29.04
55.8	4866	26.53	26.69	27.01	27.35	27.69	28.05	28.41	28.78	29.18
56.0	4873	26.65	26.81	27.14	27.48	27.82	28.18	28.54	28.92	29.31
56.2	4880	26.78	26.93	27.26	27.60	27.94	28.31	28.68	29.05	29.45
56.4	4888	26.90	27.05	27.38	27.73	28.07	28.44	28.81	29.19	29.58
56.6	4895	27.02	27.18	27.51	27.85	28.20	28.56	28.94	29.32	29.72
56.8	4903	27.14	27.30	27.63	27.98	28.33	28.69	29.07	29.46	29.86
57.0	4910	27.26	27.42	27.75	28.10	28.46	28.82	29.20	29.59	29.99
57.2	4918	27.38	27.54	27.88	28.23	28.59	28.95	29.34	29.73	30.13
57.4	4925	27.50	27.66	28.00	28.35	28.72	29.08	29.47	29.86	30.27
57.6	4932	27.62	27.79	28.13	28.48	28.85	29.21	29.60	30.00	30.41
57.8	4940	27.75	27.91	28.25	28.60	28.97	29.34	29.73	30.14	30.55

SCALE READING	INDEX OF REFRACTION	17.5° C.	18° C.	19° C.	20° C.	21° C.	22° C.	23° C.	24° C.	25° C.
		Per cent by volume	Per cent by volume	Per cent by volume	Per cent by volume	Per cent by volume	Per cent by volume	Per cent by volume	Per cent by volume	Per cent by volume
58.0	1.34947	27.87	28.03	28.38	28.73	29.10	29.47	29.87	30.27	30.69
58.2	4954	27.99	28.15	28.50	28.86	29.23	29.60	29.99	30.41	30.83
58.4	4962	28.11	28.28	28.62	28.98	29.36	29.73	30.13	30.54	30.97
58.6	4969	28.23	28.40	28.75	29.11	29.48	29.86	30.26	30.68	31.11
58.8	4977	28.35	28.52	28.88	29.23	29.61	29.99	30.40	30.82	31.25
59.0	4984	28.47	28.64	29.00	29.36	29.74	30.13	30.53	30.95	31.40
59.2	4991	28.59	28.77	29.12	29.49	29.87	30.26	30.67	31.09	31.54
59.4	4999	28.71	28.89	29.25	29.61	29.99	30.39	30.81	31.23	31.68
59.6	5006	28.84	29.01	29.37	29.74	30.13	30.53	30.94	31.38	31.83
59.8	5014	28.96	29.13	29.50	29.87	30.26	30.66	31.08	31.52	31.97
60.0	5021	29.08	29.26	29.62	29.99	30.39	30.79	31.22	31.66	32.12
60.2	5028	29.20	29.38	29.74	30.12	30.52	30.93	31.36	31.80	32.27
60.4	5036	29.32	29.50	29.87	30.25	30.65	31.06	31.50	31.94	32.41
60.6	5043	29.45	29.63	29.99	30.38	30.78	31.20	31.64	32.09	32.56
60.8	5050	29.57	29.75	30.12	30.51	30.91	31.33	31.78	32.23	32.71
61.0	5058	29.69	29.87	30.25	30.64	31.05	31.47	31.92	32.38	32.86
61.2	5065	29.81	29.99	30.38	30.77	31.18	31.61	32.06	32.52	33.01
61.4	5073	29.93	30.12	30.50	30.90	31.32	31.74	32.20	32.67	33.16
61.6	5080	30.06	30.25	30.63	31.03	31.45	31.88	32.34	32.81	33.31
61.8	5087	30.18	30.37	30.76	31.16	31.59	32.01	32.49	32.96	33.46
62.0	5095	30.31	30.50	30.89	31.29	31.72	32.16	32.63	33.10	33.60
62.2	5102	30.43	30.63	31.01	31.43	31.86	32.30	32.77	33.25	33.75
62.4	5110	30.56	30.75	31.14	31.56	31.99	32.44	32.91	33.40	33.90
62.6	5117	30.69	30.88	31.28	31.69	32.13	32.58	33.06	33.55	34.05
62.8	5124	30.81	31.01	31.41	31.83	32.27	32.72	33.20	33.70	34.21
63.0	5132	30.94	31.14	31.54	31.96	32.41	32.87	33.35	33.84	34.36
63.2	5139	31.06	31.26	31.67	32.10	32.55	33.01	33.50	33.99	34.52
63.4	5146	31.19	31.39	31.80	32.23	32.69	33.15	33.64	34.15	34.67
63.6	5154	31.32	31.52	31.93	32.37	32.83	33.30	33.79	34.30	34.83
63.8	5161	31.45	31.65	32.07	32.51	32.97	33.44	33.93	34.45	34.98
64.0	5168	31.58	31.78	32.20	32.65	33.11	33.59	34.08	34.61	35.15
64.2	5176	31.70	31.91	32.34	32.79	33.25	33.73	34.23	34.76	35.31
64.4	5183	31.83	32.04	32.47	32.92	33.39	33.88	34.39	34.92	35.48
64.6	5190	31.96	32.17	32.60	33.06	33.53	34.02	34.54	35.07	35.64
64.8	5198	32.09	32.30	32.74	33.20	33.67	34.17	34.69	35.23	35.80
65.0	5205	32.22	32.43	32.87	33.34	33.82	34.32	34.84	35.39	35.97
65.2	5212	32.35	32.67	33.01	33.48	33.96	34.47	34.99	35.55	36.13
65.4	5220	32.48	32.70	33.15	33.62	34.10	34.61	35.15	35.71	36.30
65.6	5227	32.61	32.83	33.28	33.76	34.25	34.76	35.30	35.87	36.46
65.8	5234	32.75	32.96	33.42	33.90	34.40	34.91	35.46	36.02	36.63
66.0	5242	32.88	33.10	33.56	34.04	34.54	35.06	35.62	36.19	36.79
66.2	5249	33.01	33.23	33.70	34.18	34.69	35.22	35.77	36.35	36.96
66.4	5256	33.14	33.37	33.84	34.33	34.84	35.38	35.93	36.52	37.13
66.6	5264	33.28	33.51	33.98	34.47	34.99	35.53	36.09	36.68	37.30
66.8	5271	33.41	33.65	34.12	34.62	35.14	35.69	36.25	36.84	37.48

## Alcohol table.—Continued.

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SCALE READING	INDEX OF REFRACTION	17.5° C.	18° C.	19° C.	20° C.	21° C.	22° C.	23° C.	24° C.	25° C.
		Per cent by volume	Per cent by volume	Per cent by volume	Per cent by volume	Per cent by volume	Per cent by volume	Per cent by volume	Per cent by volume	Per cent by volume
67.0	1.35278	33.55	33.79	34.26	34.76	35.29	35.84	36.41	37.01	37.65
67.2	5286	33.69	33.92	34.41	34.91	35.44	36.00	36.57	37.18	37.83
67.4	5293	33.82	34.06	34.55	35.05	35.60	36.16	36.73	37.35	38.00
67.6	5300	33.96	34.20	34.69	35.20	35.75	36.32	36.90	37.52	38.18
67.8	5308	34.09	34.34	34.84	35.35	35.90	36.48	37.06	37.69	38.35
68.0	5315	34.23	34.48	34.98	35.50	36.05	36.63	37.23	37.86	38.53
68.2	5322	34.36	34.62	35.13	35.65	36.21	36.79	37.39	38.03	38.70
68.4	5329	34.50	34.76	35.27	35.80	36.37	36.95	37.56	38.21	38.88
68.6	5337	34.64	34.90	35.42	35.95	36.52	37.12	37.73	38.38	39.06
68.8	5344	34.77	35.04	35.57	36.10	36.68	37.28	37.90	38.56	39.24
69.0	5351	34.91	35.19	35.71	36.25	36.84	37.45	38.07	38.73	39.43
69.2	5359	35.04	35.33	35.86	36.41	36.99	37.61	38.24	38.90	39.61
69.4	5366	35.19	35.47	36.01	36.56	37.15	37.78	38.41	39.08	39.80
69.6	5373	35.34	35.62	36.16	36.72	37.32	37.94	38.58	39.26	39.98
69.8	5381	35.49	35.76	36.31	36.87	37.48	38.11	38.75	39.45	40.17
70.0	5388	35.64	35.91	36.46	37.02	37.64	38.28	38.92	39.63	40.35
70.2	5395	35.78	36.05	36.61	37.19	37.80	38.45	39.10	39.81	40.53
70.4	5402	35.93	36.20	36.76	37.35	37.97	38.61	39.28	39.99	40.72
70.6	5410	36.08	36.35	36.92	37.51	38.13	38.78	39.46	40.17	40.90
70.8	5417	36.23	36.50	37.07	37.67	38.30	38.95	39.64	40.35	41.08
71.0	5424	36.38	36.65	37.23	37.83	38.47	39.12	39.82	40.54	41.27
71.2	5432	36.53	36.80	37.39	37.99	38.63	39.30	40.00	40.72	40.46
71.4	5439	36.68	36.95	37.55	38.16	38.80	39.48	40.18	40.90	41.64
71.6	5446	36.83	37.11	37.71	38.32	38.97	39.65	40.36	41.08	41.83
71.8	5454	36.98	37.27	37.87	38.49	39.14	39.83	40.54	41.27	42.02
72.0	5461	37.13	37.42	38.02	38.65	39.31	40.01	40.72	41.45	42.21
72.2	5468	37.29	37.58	38.19	38.82	39.49	40.18	40.90	41.64	42.40
72.4	5475	37.44	37.73	38.35	38.98	39.66	40.36	41.08	41.82	42.58
72.6	5483	37.60	37.89	38.51	39.16	39.83	40.54	41.26	42.01	42.77
72.8	5490	37.75	38.05	38.67	39.33	40.01	40.71	41.45	42.19	42.96
73.0	5497	37.91	38.21	38.84	39.50	40.18	40.88	41.63	42.38	43.15
73.2	5504	38.06	38.37	39.00	39.67	40.36	41.06	41.81	42.56	43.33
73.4	5512	38.22	38.53	39.17	39.84	40.53	41.24	41.99	42.75	43.52
73.6	5519	38.38	38.69	39.34	40.02	40.70	41.42	42.17	42.93	43.70
73.8	5526	38.54	38.85	39.50	40.19	40.88	41.60	42.36	43.12	43.89
74.0	5533	38.70	39.01	39.67	40.36	41.05	41.78	42.54	43.31	44.08
74.2	5541	38.86	39.18	39.84	40.53	41.23	41.96	42.72	43.49	44.28
74.4	5548	39.02	39.34	40.01	40.71	41.41	42.15	42.91	43.68	44.48
74.6	5555	39.18	39.51	40.18	40.88	41.59	42.33	43.09	43.86	44.67
74.8	5563	39.35	39.68	40.35	41.05	41.77	42.51	43.28	44.05	44.87
75.0	5570	39.51	39.84	40.53	41.23	41.95	42.70	43.46	44.25	45.07
75.2	5577	39.68	40.01	40.70	41.41	42.13	42.88	43.65	44.44	45.29
75.4	5584	39.84	40.18	40.87	41.58	42.31	43.07	43.83	44.63	45.50
75.6	5592	40.01	40.35	41.04	41.76	42.49	43.25	44.02	44.83	45.71
75.8	5599	40.18	40.53	41.22	41.94	42.67	43.44	44.21	45.03	45.92



SCALE READING	INDEX OF REFRACTION	17.5° C.	18° C.	19° C.	20° C.	21° C.	22° C.	23° C.	24° C.	25° C.
		Per cent by volume	Per cent by volume	Per cent by volume	Per cent by volume	Per cent by volume	Per cent by volume	Per cent by volume	Per cent by volume	Per cent by volume
76.0	1.35606	40.35	40.70	41.40	42.12	42.85	43.63	44.41	45.24	46.12
76.2	5613	40.53	40.87	41.57	42.30	43.04	43.81	44.60	45.44	46.34
76.4	5621	40.70	41.04	41.75	42.48	43.22	44.00	44.80	45.65	46.56
76.6	5628	40.87	41.22	41.92	42.66	43.41	44.19	44.99	45.86	46.78
76.8	5635	41.04	41.39	42.10	42.84	43.60	44.38	45.19	46.07	47.00
77.0	5642	41.22	41.57	42.28	43.02	43.79	44.57	45.40	46.29	47.23
77.2	5650	41.39	41.74	42.46	43.20	43.97	44.76	45.60	46.51	47.45
77.4	5657	41.57	41.91	42.63	43.39	44.16	44.95	45.81	46.73	47.68
77.6	5664	41.75	42.09	42.81	43.57	44.35	45.15	46.01	46.95	47.91
77.8	5671	41.92	42.26	42.99	43.76	44.54	45.35	46.23	47.17	48.14
78.0	5678	42.09	42.43	43.17	43.94	44.73	45.56	46.45	47.40	48.37
78.2	5686	42.26	42.61	43.36	44.13	44.92	45.76	46.67	47.63	48.60
78.4	5693	42.44	42.78	43.54	44.32	45.12	45.96	46.89	47.85	48.84
78.6	5700	42.61	42.96	43.72	44.51	45.32	46.17	47.11	48.08	49.07
78.8	5707	42.78	43.14	43.91	44.70	45.52	46.39	47.34	48.31	49.31
79.0	5715	42.95	43.32	44.09	44.89	45.72	46.61	47.56	48.53	49.54
79.2	5722	43.13	43.50	44.28	45.08	45.92	46.83	47.79	48.76	49.77
79.4	5729	43.31	43.68	44.47	45.28	46.13	47.04	48.01	48.99	50.01
79.6	5736	43.49	43.86	44.65	45.48	46.34	47.26	48.23	49.22	50.24
79.8	5744	43.67	44.05	44.84	45.68	46.56	47.48	48.46	49.45	50.48
80.0	5751	43.85	44.24	45.04	45.88	46.77	47.70	48.68	49.68	50.71

Percentages by weight, corresponding to various percentages by volume at 21  
15.56°C. (60°F.) in mixtures of ethyl alcohol and water.<sup>1</sup>

PER CENT ALCOHOL BY VOLUME AT 60° F.	PER CENT ALCOHOL BY WEIGHT	DIFFERENCES	PER CENT ALCOHOL BY VOLUME AT 60° F.	PER CENT ALCOHOL BY WEIGHT	DIFFERENCES
0	0.000		50	42.487	
1	0.795	0.795	51	43.428	0.941
2	1.593	.798	52	44.374	.946
3	2.392	.799	53	45.326	.952
4	3.194	.802	54	46.283	.957
		.804			.962
5	3.998		55	47.245	
6	4.804	.806	56	48.214	.969
7	5.612	.808	57	49.187	.973
8	6.422	.810	58	50.167	.980
9	7.234	.812	59	51.154	.987
		.813			.993
10	8.047		60	52.147	
11	8.862	.815	61	53.146	.999
12	9.679	.817	62	54.152	1.006
13	10.497	.818	63	55.165	1.013
14	11.317	.820	64	56.184	1.019
		.821			1.024
15	12.138		65	57.208	
16	12.961	.823	66	58.241	1.033
17	13.786	.825	67	59.279	1.038
18	14.612	.826	68	60.325	1.046
19	15.440	.828	69	61.379	1.054
		.829			1.062
20	16.269		70	62.441	
21	17.100	.831	71	63.511	1.070
22	17.933	.833	72	64.588	1.077
23	18.768	.835	73	65.674	1.086
24	19.604	.836	74	66.768	1.094
		.839			1.102
25	20.443		75	67.870	
26	21.285	.842	76	68.982	1.112
27	22.127	.842	77	70.102	1.120
28	22.973	.846	78	71.234	1.132
29	23.820	.847	79	72.375	1.141
		.850			1.151
30	24.670		80	73.526	
31	25.524	.854	81	74.686	1.160
32	26.382	.858	82	75.858	1.172
33	27.242	.860	83	77.039	1.181
34	28.104	.862	84	78.233	1.194
		.867			1.208
35	28.971		85	79.441	
36	29.842	.871	86	80.662	1.221
37	30.717	.875	87	81.897	1.235
38	31.596	.879	88	83.144	1.247
39	32.478	.882	89	84.408	1.264
		.886			1.281
40	33.364		90	85.689	
41	34.254	.890	91	86.989	1.300
42	35.150	.896	92	88.310	1.321
43	36.050	.900	93	89.652	1.342
44	36.955	.905	94	91.025	1.373
		.910			1.398
45	37.865		95	92.423	
46	38.778	.913	96	93.851	1.428
47	39.697	.919	97	95.315	1.464
48	40.622	.925	98	96.820	1.505
49	41.551	.929	99	98.381	1.561
		.936			1.619
50	42.487		100	100.000	

<sup>1</sup> Bureau of Standards Circular No. 10, p. 18 (1924).

22 For determining added water in milk by means of the freezing-point depression  
(based on *Winter's table*<sup>1</sup>).

(For practical purposes the added water results may be expressed to the nearest decimal.)

FREEZING POINT OF SAMPLE, BELOW ZERO C.	ADDED WATER, PER CENT BY VOLUME	FREEZING POINT OF SAMPLE, BELOW ZERO C.	ADDED WATER, PER CENT BY VOLUME	FREEZING POINT OF SAMPLE, BELOW ZERO C.	ADDED WATER, PER CENT BY VOLUME
0.550	0.00	0.505	8.18	0.460	16.36
.549	0.18	.504	8.36	.459	16.54
.548	0.36	.503	8.54	.458	16.73
.547	0.54	.502	8.73	.457	16.91
.546	0.73	.501	8.91	.456	17.09
.545	0.91	.500	9.09	.455	17.27
.544	1.09	.499	9.27	.454	17.45
.543	1.27	.498	9.45	.453	17.64
.542	1.45	.497	9.64	.452	17.82
.541	1.63	.496	9.82	.451	18.00
.540	1.82	.495	10.00	.450	18.18
.539	2.00	.494	10.18	.449	18.36
.538	2.18	.493	10.36	.448	18.54
.537	2.36	.492	10.54	.447	18.73
.536	2.54	.491	10.72	.446	18.91
.535	2.72	.490	10.91	.445	19.09
.534	2.91	.489	11.09	.444	19.27
.533	3.09	.488	11.27	.443	19.45
.532	3.27	.487	11.45	.442	19.64
.531	3.45	.486	11.64	.441	19.82
.530	3.64	.485	11.82	.440	20.00
.529	3.82	.484	12.00	.439	20.18
.528	4.00	.483	12.18	.438	20.36
.527	4.18	.482	12.36	.437	20.54
.526	4.36	.481	12.54	.436	20.73
.525	4.54	.480	12.73	.435	20.91
.524	4.73	.479	12.91	.434	21.09
.523	4.91	.478	13.09	.433	21.27
.522	5.09	.477	13.27	.432	21.45
.521	5.27	.476	13.45	.431	21.64
.520	5.45	.475	13.64	.430	21.82
.519	5.63	.474	13.82	.429	22.00
.518	5.82	.473	14.00	.428	22.18
.517	6.00	.472	14.18	.427	22.36
.516	6.18	.471	14.37	.426	22.54
.515	6.36	.470	14.54	.425	22.73
.514	6.54	.469	14.73	.424	22.91
.513	6.73	.468	14.91	.423	23.09
.512	6.91	.467	15.09	.422	23.27
.511	7.09	.466	15.27	.421	23.45
.510	7.27	.465	15.45	.420	23.64
.509	7.45	.464	15.63	.419	23.82
.508	7.64	.463	15.82	.418	24.00
.507	7.82	.462	16.00	.417	24.18
.506	8.00	.461	16.18	.416	24.36

<sup>1</sup> *Chem. News*, 110, 283 (1914).

REFERENCE TABLES

XLII

*For determining added water in milk by means of the freezing-point depression 22  
(based on Winter's table).—Concluded.*

FREEZING POINT OF SAMPLE, BELOW ZERO C.	ADDED WATER, PER CENT BY VOLUME	FREEZING POINT OF SAMPLE, BELOW ZERO C.	ADDED WATER, PER CENT BY VOLUME	FREEZING POINT OF SAMPLE, BELOW ZERO C.	ADDED WATER, PER CENT BY VOLUME
0.415	24.54	0.390	29.09	0.365	33.64
.414	24.73	.389	29.27	.364	33.82
.413	24.91	.388	29.45	.363	34.00
.412	25.09	.387	29.64	.362	34.18
.411	25.27	.386	29.82	.361	34.36
.410	25.45	.385	30.00	.360	34.54
.409	25.64	.384	30.18	.359	34.73
.408	25.82	.383	30.36	.358	34.91
.407	26.00	.382	30.54	.357	35.09
.406	26.18	.381	30.73	.356	35.27
.405	26.36	.380	30.91	.355	35.45
.404	26.54	.379	31.09	.354	35.64
.403	26.73	.378	31.27	.353	35.82
.402	26.91	.377	31.45	.352	36.00
.401	27.09	.376	31.64	.351	36.18
.400	27.27	.375	31.82	.350	36.36
.399	27.45	.374	32.00		
.398	27.64	.373	32.18		
.397	27.82	.372	32.36		
.396	28.00	.371	32.54		
.395	28.18	.370	32.73		
.394	28.36	.369	32.91		
.393	28.54	.368	33.09		
.392	28.73	.367	33.27		
.391	28.91	.366	33.45		

## 23

*Density of carbon dioxide (Parr).<sup>1</sup>*

(Weight in milligrams of 1 cc of carbon dioxide at 700–770 mm pressure and 10–30°C. Corrected for aqueous vapor and barometer readings on glass scale. Calculated from 1.976 equals weight of liter of CO<sub>2</sub> at 0°C., 760 mm pressure and 41° latitude.)

mm.	10°	11°	12°	13°	14°	15°	16°	17°	18°	19°
700	1.7288	1.7201	1.7113	1.7020	1.6927	1.6833	1.6739	1.6716	1.6632	1.6547
702	.7338	.7252	.7164	.7072	.6980	.6884	.6848	.6765	.6680	.6595
704	.7388	.7302	.7215	.7124	.7033	.6935	.6897	.6813	.6729	.6644
706	.7438	.7352	.7265	.7175	.7084	.7019	.6946	.6862	.6778	.6692
708	.7488	.7403	.7317	.7228	.7139	.7067	.6995	.6911	.6826	.6741
710	.7538	.7453	.7368	.7280	.7192	.7118	.7044	.6960	.6874	.6789
712	.7588	.7504	.7419	.7332	.7245	.7169	.7092	.7008	.6922	.6837
714	.7638	.7555	.7470	.7384	.7298	.7220	.7141	.7057	.6970	.6886
716	.7688	.7605	.7521	.7436	.7351	.7271	.7190	.7106	.7019	.6934
718	.7738	.7656	.7572	.7488	.7404	.7322	.7239	.7154	.7068	.6983
720	.7788	.7706	.7623	.7540	.7457	.7373	.7288	.7203	.7117	.7031
722	.7838	.7756	.7673	.7590	.7506	.7422	.7337	.7252	.7166	.7079
724	.7888	.7806	.7723	.7639	.7555	.7471	.7386	.7301	.7215	.7128
726	.7938	.7856	.7773	.7689	.7605	.7520	.7435	.7349	.7263	.7176
728	.7988	.7905	.7822	.7738	.7654	.7569	.7484	.7398	.7312	.7225
730	.8038	.7955	.7872	.7788	.7703	.7618	.7533	.7447	.7360	.7273
732	.8089	.8005	.7921	.7837	.7752	.7667	.7582	.7496	.7409	.7321
734	.8139	.8055	.7971	.7887	.7802	.7717	.7631	.7545	.7458	.7370
736	.8189	.8105	.8021	.7936	.7851	.7766	.7680	.7593	.7506	.7418
738	.8239	.8155	.8071	.7986	.7901	.7815	.7729	.7642	.7555	.7467
740	.8288	.8204	.8120	.8035	.7950	.7864	.7778	.7691	.7603	.7515
742	.8338	.8254	.8170	.8085	.7999	.7913	.7827	.7740	.7652	.7564
744	.8388	.8304	.8219	.8134	.8048	.7962	.7875	.7788	.7700	.7612
746	.8439	.8354	.8269	.8184	.8098	.8011	.7924	.7837	.7749	.7661
748	.8489	.8404	.8319	.8233	.8147	.8060	.7973	.7886	.7798	.7709
750	.8539	.8454	.8368	.8282	.8196	.8109	.8022	.7934	.7846	.7757
752	.8589	.8504	.8418	.8332	.8245	.8159	.8072	.7984	.7895	.7806
754	.8639	.8554	.8468	.8382	.8295	.8208	.8120	.8032	.7944	.7854
756	.8689	.8603	.8517	.8431	.8344	.8257	.8169	.8081	.7992	.7902
758	.8739	.8653	.8567	.8481	.8394	.8306	.8218	.8130	.8041	.7951
760	.8789	.8703	.8617	.8530	.8443	.8355	.8267	.8178	.8089	.7999
762	.8839	.8753	.8667	.8580	.8492	.8404	.8316	.8227	.8138	.8048
764	.8890	.8803	.8716	.8629	.8541	.8453	.8365	.8276	.8187	.8096
766	.8940	.8853	.8765	.8677	.8591	.8503	.8414	.8325	.8235	.8144
768	.8990	.8903	.8816	.8728	.8640	.8552	.8463	.8374	.8284	.8193
770	.9040	.8953	.8865	.8777	.8689	.8601	.8512	.8422	.8332	.8241

<sup>1</sup> *J. Am. Chem. Soc.*, 31, 237 (1909). The values of 700–718 mm. have been calculated by formula given by Parr.

*Density of carbon dioxide (Parr).—Concluded.*

23

20°	21°	22°	23°	24°	25°	26°	27°	28°	29°	30°	mm.
1.8462	1.8370	1.8278	1.8195	1.8112	1.8021	1.5930	1.5837	1.5744	1.5649	1.5554	700
.8510	.8419	.8327	.8243	.8160	.8068	.5977	.5884	.5791	.5698	.5600	702
.8558	.8467	.8376	.8292	.8207	.8116	.6025	.5931	.5838	.5742	.5647	704
.8607	.8516	.8425	.8340	.8254	.8163	.6072	.5979	.5885	.5789	.5693	706
.8655	.8564	.8474	.8388	.8302	.8211	.6119	.6026	.5932	.5836	.5740	708
.8703	.8613	.8522	.8436	.8350	.8258	.6166	.6073	.5978	.5882	.5786	710
.8751	.8662	.8571	.8485	.8397	.8305	.6214	.6120	.6025	.5929	.5832	712
.8799	.8710	.8620	.8533	.8444	.8353	.6261	.6167	.6072	.5976	.5879	714
.8848	.8759	.8670	.8581	.8492	.8400	.6308	.6215	.6119	.6023	.5925	716
.8896	.8807	.8718	.8629	.8540	.8448	.6356	.6262	.6166	.6069	.5972	718
.8944	.8856	.8767	.8678	.8587	.8495	.6403	.6309	.6213	.6116	.6018	720
.8992	.8904	.8815	.8726	.8635	.8543	.6450	.6356	.6260	.6163	.6065	722
.9041	.8953	.8863	.8773	.8682	.8590	.6497	.6403	.6307	.6210	.6111	724
.9089	.9001	.8911	.8821	.8730	.8638	.6544	.6450	.6354	.6256	.6157	726
.9137	.9049	.8959	.8869	.8778	.8685	.6591	.6497	.6401	.6303	.6204	728
.9185	.9097	.9007	.8917	.8825	.8732	.6638	.6544	.6448	.6350	.6251	730
.9233	.9145	.9055	.8964	.8873	.8779	.6685	.6591	.6494	.6396	.6297	732
.9282	.9193	.9103	.9012	.8920	.8827	.6733	.6638	.6541	.6443	.6343	734
.9330	.9241	.9151	.9060	.8968	.8875	.6780	.6685	.6588	.6490	.6390	736
.9378	.9289	.9199	.9107	.9015	.8922	.6827	.6732	.6635	.6537	.6437	738
.9426	.9337	.9247	.9155	.9063	.8969	.6874	.6778	.6681	.6583	.6483	740
.9475	.9385	.9295	.9203	.9111	.9017	.6922	.6825	.6729	.6630	.6530	742
.9523	.9433	.9342	.9250	.9158	.9064	.6969	.6873	.6776	.6677	.6577	744
.9571	.9481	.9390	.9298	.9206	.9112	.7016	.6920	.6822	.6723	.6623	746
.9619	.9529	.9438	.9346	.9253	.9159	.7063	.6967	.6869	.6770	.6670	748
.9667	.9577	.9486	.9394	.9301	.9208	.7110	.7014	.6916	.6817	.6716	750
.9716	.9625	.9534	.9441	.9348	.9254	.7158	.7061	.6963	.6864	.6763	752
.9764	.9673	.9582	.9489	.9396	.9301	.7205	.7108	.7010	.6910	.6809	754
.9812	.9721	.9630	.9537	.9443	.9348	.7253	.7155	.7057	.6957	.6856	756
.9861	.9770	.9678	.9585	.9491	.9396	.7300	.7202	.7104	.7004	.6903	758
.9909	.9818	.9725	.9632	.9538	.9443	.7347	.7249	.7150	.7050	.6949	760
.9957	.9866	.9773	.9680	.9586	.9490	.7394	.7296	.7197	.7097	.6996	762
.8005	.7914	.7821	.7728	.7633	.7538	.7441	.7343	.7244	.7144	.7042	764
.8053	.7962	.7869	.7776	.7681	.7585	.7488	.7390	.7291	.7191	.7089	766
.8102	.8010	.7917	.7823	.7728	.7633	.7535	.7437	.7338	.7237	.7135	768
.8150	.8058	.7965	.7871	.7776	.7680	.7582	.7484	.7385	.7284	.7182	770

24 Correction factors for the gasometric determination of carbon dioxide.<sup>1</sup>

(Based on sample weighing 1.7000 grams.)

°C.	15.0°	15.5°	16.0°	16.5°	17.0°	17.5°	18.0°	18.5°	
mm.									inches
700	0.99194	0.99006	0.98818	0.98573	0.98329	0.98082	0.97835	0.97585	27.56
702	.99494	.99300	.99106	.98862	.98618	.98368	.98118	.97868	27.64
704	.99794	.99544	.99394	.99147	.98900	.98653	.98406	.98156	27.72
706	1.00094	.99896	.99682	.99435	.99188	.98941	.98694	.98446	27.80
708	.00394	1.00183	.99971	.99723	.99476	.99226	.98976	.98726	27.87
710	.00694	.00477	1.00259	1.00012	.99765	.99512	.99259	.99009	27.95
712	.00994	.00767	.00541	.00294	1.00047	.99795	.99541	.99291	28.03
714	.01294	.01061	.00829	.00582	.00335	1.00080	.99824	.99570	28.11
716	.01594	.01356	.01118	.00871	.00624	.00368	1.00112	.99861	28.19
718	.01894	.01650	.01406	.01156	.00906	.00653	.00400	1.00150	28.27
720	.02194	.01949	.01694	.01444	.01194	.00941	.00688	.00435	28.35
722	.02482	.02232	.01982	.01732	.01483	.01229	.00976	.00720	28.43
724	.02771	.02521	.02271	.02021	.01771	.01518	.01265	.01009	28.50
726	.03059	.02809	.02559	.02306	.02053	.01800	.01574	.01291	28.58
728	.03347	.03097	.02847	.02594	.02341	.02088	.01835	.01580	28.66
730	.03635	.03385	.03135	.02882	.02629	.02374	.02118	.01862	28.74
732	.03924	.03674	.03424	.03171	.02918	.02662	.02406	.02147	28.82
734	.04218	.03915	.03712	.03459	.03206	.02950	.02694	.02435	28.90
736	.04506	.04253	.04000	.03744	.03488	.03232	.02976	.02718	28.98
738	.04794	.04541	.04288	.04037	.03776	.03521	.03265	.03006	29.06
740	.05082	.04829	.04576	.04321	.04065	.03806	.03547	.03288	29.13
742	.05371	.05118	.04865	.04609	.04353	.04094	.03835	.03577	29.21
744	.05659	.05403	.05147	.04991	.04635	.04377	.04118	.03859	29.29
746	.05947	.05691	.05435	.05180	.04924	.04665	.04406	.04147	29.37
748	.06235	.05979	.05724	.05418	.05212	.04953	.04694	.04432	29.45
750	.06524	.06218	.06012	.05748	.05494	.05235	.04976	.04715	29.53
752	.06818	.06512	.06306	.06047	.05788	.05527	.05265	.05003	29.61
754	.07106	.06847	.06588	.06330	.06071	.05812	.05553	.05289	29.69
756	.07394	.07135	.06876	.06618	.06359	.06197	.05835	.05571	29.76
758	.07682	.07423	.07165	.06906	.06647	.06386	.06124	.05859	29.84
760	.07971	.07712	.07453	.07191	.06929	.06668	.06406	.06141	29.92
762	.08259	.08050	.07741	.07480	.07218	.06956	.06694	.06430	30.00
764	.08547	.08288	.08029	.07768	.07506	.07244	.06982	.06715	30.08
766	.08841	.08580	.08318	.08056	.07794	.07530	.07265	.06997	30.16
768	.09129	.08868	.08606	.08344	.08082	.07818	.07553	.07285	30.24
770	.09418	.09156	.08894	.08630	.08365	.08100	.07835	.07567	30.31
°F.	59.0°	59.9°	60.8°	61.7°	62.6°	63.5°	64.4°	65.3°	

<sup>1</sup> Calculated from 1.976 = weight of 1 liter CO<sub>2</sub> at 0°C., 760 mm pressure and 41° latitude. Formula given S. W. Parr, *J. Am. Chem. Soc.*, 31: 237 (1909).

## Correction factors for the gasometric determination of carbon dioxide.—Continued. 24

°C.	18.0°	19.5°	20.0°	20.5°	21.0°	21.5°	22.0°	22.5°	
mm.	0.97335	0.97085	0.96835	0.96584	0.96294	0.96023	0.95753	0.95509	inches
700	.97018	.97368	.97118	.96850	.96582	.96311	.96041	.95794	27.56
702	.97906	.97653	.97400	.97132	.96865	.96597	.96329	.96082	27.64
704	.98188	.97938	.97688	.97420	.97153	.96888	.96624	.96371	27.72
706	.98476	.98224	.97971	.97703	.97435	.97173	.96912	.96656	27.80
708	.98759	.98506	.98253	.97998	.97724	.97459	.97195	.96938	27.87
710									27.95
712	.99041	.98788	.98535	.98273	.98012	.97747	.97483	.97227	28.03
714	.99329	.99073	.98818	.98556	.98294	.98032	.97771	.97512	28.11
716	.99612	.99358	.99105	.98844	.98582	.98323	.98065	.97800	28.19
718	.99900	.99644	.99388	.99126	.98865	.98606	.98348	.98083	28.27
720	1.00182	.99925	.99671	.99412	.99153	.98894	.98636	.98371	28.35
722	.00465	1.00209	.99953	.99694	.99435	.99176	.98918	.98653	28.43
724	.00753	.00497	1.00241	.99982	.99724	.99462	.99200	.98932	28.50
726	.01035	.00779	.00524	1.00285	1.00026	.99764	.99503	.99245	28.58
728	.01324	.01065	.00806	.00547	.00288	1.00027	.99765	.99507	28.66
730	.01606	.01347	.01088	.00829	.00571	.00306	1.00041	.99781	28.74
732	.01888	.01629	.01371	.01112	.00853	.00598	.00324	1.00066	28.82
734	.02176	.01919	.01659	.01407	.01155	.00907	.00650	.00385	28.90
736	.02459	.02200	.01941	.01679	.01418	.01153	.00898	.00620	28.98
738	.02747	.02486	.02224	.01962	.01700	.01435	.01171	.00900	29.06
740	.03029	.02768	.02506	.02244	.01982	.01717	.01453	.01182	29.13
742	.03318	.03056	.02794	.02529	.02265	.02000	.01735	.01464	29.21
744	.03600	.03338	.03076	.02811	.02547	.02279	.02012	.01752	29.29
746	.03888	.03624	.03359	.03094	.02829	.02561	.02294	.02024	29.37
748	.04171	.03906	.03641	.03376	.03112	.02844	.02576	.02306	29.45
750	.04453	.04189	.03924	.03659	.03394	.03126	.02859	.02589	29.53
752	.04741	.04477	.04212	.03944	.03676	.03408	.03141	.02868	29.61
754	.05024	.04759	.04494	.04226	.03959	.03691	.03424	.03156	29.69
756	.05306	.05041	.04776	.04508	.04241	.03973	.03706	.03433	29.76
758	.05594	.05329	.05065	.04797	.04529	.04259	.03988	.03715	29.84
760	.05876	.05612	.05347	.05079	.04812	.04543	.04265	.03992	29.92
762	.06165	.05897	.05629	.05361	.05094	.04821	.04547	.04274	30.00
764	.06447	.06179	.05912	.05644	.05376	.05103	.04829	.04556	30.08
766	.06729	.06462	.06194	.05926	.05659	.05386	.05112	.04839	30.16
768	.07018	.06750	.06482	.06212	.05941	.05668	.05394	.05118	30.24
770	.07300	.07032	.06765	.06494	.06224	.05950	.05676	.05400	30.31
°F.	66.2°	67.1°	68.0°	68.9°	69.8°	70.7°	71.6°	72.5°	



24 Correction factors for the gasometric determination of carbon dioxide.—Continued.

°C.	23.0°	23.5°	24.0°	24.5°	25.0°	25.5°	26.0°	26.5°	
mm.									inches
700	0.95265	0.95020	0.94776	0.94508	0.94241	0.93973	0.93706	0.93432	27.56
702	.95547	.95303	.95059	.94788	.94518	.94250	.93982	.93708	27.64
704	.95835	.95585	.95335	.95067	.94790	.94522	.94255	.93988	27.72
706	.96118	.95865	.95612	.95344	.95076	.94808	.94541	.94275	27.80
708	.96400	.96147	.95894	.95626	.95359	.95088	.94818	.94544	27.87
710	.96682	.96429	.96176	.95905	.95635	.95364	.95094	.94820	27.95
712	.96971	.96712	.96453	.96182	.95912	.95644	.95376	.95100	28.03
714	.97253	.96991	.96729	.96461	.96194	.95923	.95655	.95376	28.11
716	.97535	.97273	.97012	.96741	.96471	.96200	.95929	.95655	28.19
718	.97818	.97556	.97294	.97023	.96753	.96482	.96212	.95935	28.27
720	.98106	.97838	.97571	.97300	.97029	.96758	.96488	.96213	28.35
722	.98388	.98120	.97853	.97582	.97312	.97038	.96765	.96488	28.43
724	.98665	.98397	.98129	.97858	.97588	.97314	.97041	.96764	28.50
726	.98947	.98679	.98412	.98141	.97871	.97594	.97318	.97041	28.58
728	.99229	.98961	.98694	.98420	.98147	.97870	.97594	.97319	28.66
730	.99512	.99241	.98971	.98697	.98424	.98147	.97871	.97594	28.74
732	.99788	.99517	.99247	.98973	.98700	.98423	.98147	.97871	28.82
734	1.00071	.99799	.99529	.99255	.98982	.98705	.98429	.98155	28.90
736	.00353	1.00083	.99812	.99538	.99265	.98985	.98706	.98428	28.98
738	.00629	.00359	1.00088	.99815	.99541	.99261	.98982	.98703	29.06
740	.00912	.00643	.00371	1.00095	.99818	.99538	.99259	.98975	29.13
742	.01194	.00923	.00653	.00377	1.00100	.99820	.99541	.99258	29.21
744	.01471	.01200	.00929	.00643	.00376	1.00097	.99818	.99535	29.29
746	.01753	.01482	.01212	.00936	.00659	.00376	1.00094	.99809	29.37
748	.02035	.01762	.01488	.01212	.00935	.00653	.00371	1.00098	29.45
750	.02318	.02045	.01771	.01492	.01212	.00936	.00659	.00370	29.53
752	.02594	.02321	.02047	.01771	.01494	.01211	.00929	.00644	29.61
754	.02876	.02603	.02329	.02050	.01771	.01483	.01206	.00921	29.69
756	.03159	.02883	.02606	.02326	.02047	.01764	.01482	.01197	29.76
758	.03441	.03165	.02889	.02608	.02329	.02047	.01765	.01477	29.84
760	.03718	.03442	.03165	.02889	.02606	.02323	.02041	.01753	29.92
762	.04000	.03724	.03447	.03164	.02882	.02600	.02318	.02030	30.00
764	.04282	.04003	.03723	.03444	.03165	.02880	.02594	.02306	30.08
766	.04565	.04285	.04005	.03723	.03441	.03156	.02871	.02583	30.16
768	.04841	.04562	.04282	.04003	.03724	.03435	.03147	.02850	30.24
770	.05123	.04844	.04564	.04282	.04000	.03712	.03424	.03136	30.31
°F.	73.4°	74.3°	75.2°	76.1°	77.0°	77.9°	78.8°	79.7°	

REFERENCE TABLES

XLIJ

*Correction factors for the gasometric determination of carbon dioxide.—Continued. 24*

°C.	27.0°	27.5°	28.0°	28.5°	29.0°	29.5°	30.0°	30.5°	
mm.									<i>inches</i>
700	0.93159	0.92885	0.92612	0.92332	0.92053	0.91773	0.91494	0.91203	27.55
702	.93435	.93161	.92888	.92608	.92329	.92047	.91765	.91476	27.64
704	.93712	.93438	.93165	.92882	.92600	.92320	.92041	.91750	27.72
706	.93994	.93717	.93441	.93158	.92876	.92594	.92312	.92024	27.80
708	.94271	.93994	.93718	.93435	.93153	.92870	.92588	.92297	27.87
710	.94547	.94267	.93988	.93706	.93424	.93141	.92859	.92567	27.95
712	.94824	.94544	.94265	.93982	.93700	.93414	.93129	.92841	28.03
714	.95100	.94820	.94541	.94258	.93976	.93691	.93406	.93115	28.11
716	.95382	.95100	.94818	.94535	.94253	.93964	.93676	.93388	28.19
718	.95659	.95376	.95094	.94809	.94524	.94238	.93953	.93662	28.27
720	.95939	.95655	.95371	.95085	.94800	.94512	.94224	.93932	28.35
722	.96212	.95929	.95647	.95361	.95076	.94788	.94500	.94209	28.43
724	.96488	.96206	.95924	.95638	.95353	.95062	.94771	.94479	28.50
726	.96765	.96482	.96200	.95912	.95624	.95332	.95041	.94750	28.58
728	.97041	.96758	.96476	.96188	.95900	.95609	.95318	.95029	28.66
730	.97318	.97036	.96753	.96464	.96176	.95885	.95594	.95300	28.74
732	.97594	.97309	.97024	.96735	.96447	.96156	.95865	.95578	28.82
734	.97871	.97585	.97300	.97012	.96724	.96429	.96135	.95844	28.90
736	.98147	.97861	.97576	.97288	.97000	.96706	.96412	.96118	28.98
738	.98424	.98138	.97853	.97564	.97276	.96982	.96688	.96394	29.06
740	.98699	.98409	.98124	.97835	.97547	.97253	.96959	.96665	29.13
742	.98976	.98681	.98406	.98115	.97824	.97529	.97235	.96941	29.21
744	.99253	.98967	.98682	.98391	.98100	.97806	.97512	.97215	29.29
746	.99529	.99241	.98953	.98662	.98371	.98076	.97782	.97485	29.37
748	.99806	.99517	.99229	.98938	.98647	.98353	.98059	.97762	29.45
750	1.00082	.99796	.99506	.99215	.98924	.98636	.98329	.98032	29.53
752	.00359	1.00071	.99782	.99491	.99200	.98903	.98606	.98306	29.61
754	.00635	.00342	1.00059	.99738	.99471	.99173	.98876	.98579	29.69
756	.00912	.00624	.00335	1.00041	.99747	.99450	.99153	.98853	29.76
758	.01188	.00900	.00612	.00318	1.00024	.99724	.99429	.99129	29.84
760	.01465	.01174	.00882	.00588	.00294	.99995	.99700	.99400	29.92
762	.01741	.01450	.01159	.00865	.00571	1.00274	.99976	.99673	30.00
764	.02018	.01727	.01435	.01141	.00847	.00547	1.00247	.99948	30.08
766	.02294	.02003	.01712	.01418	.01124	.00824	.00524	1.00221	30.16
768	.02571	.02280	.01988	.01694	.01394	.01094	.00794	.00494	30.24
770	.02847	.02556	.02265	.01968	.01671	.01371	.01071	.00768	30.31
°F.	80.6°	81.5°	82.4°	83.3°	84.2°	85.1°	86.0°	86.9°	

## 24 Correction factors for the gasometric determination of carbon dioxide.—Concluded.

°C.	31.0°	31.5°	32.0°	32.5°	33.0°	33.5°	34.0°	34.5°	35.0°	inches
700	0.90912	0.90620	0.90329	0.90082	0.89735	0.89432	0.89129	0.88821	0.88512	27.56
702	.91188	.90894	.90600	.90303	.90006	.89703	.89400	.89091	.88782	27.64
704	.91459	.91165	.90871	.90576	.90282	.89976	.89671	.89362	.89053	27.72
706	.91735	.91441	.91147	.90847	.90547	.90241	.89935	.89627	.89318	27.80
708	.92006	.91712	.91418	.91118	.90818	.90512	.90206	.89897	.89588	27.87
710	.92276	.91982	.91688	.91388	.91088	.90782	.90476	.90168	.89859	27.95
712	.92553	.92256	.91959	.91659	.91359	.91053	.90747	.90438	.90129	28.03
714	.92824	.92529	.92235	.91932	.91629	.91323	.91018	.90706	.90394	28.11
716	.93100	.92803	.92506	.92203	.91900	.91594	.91288	.90976	.90665	28.19
718	.93371	.93073	.92776	.92474	.92171	.91865	.91559	.91247	.90935	28.27
720	.93641	.93344	.93047	.92744	.92441	.92135	.91829	.91517	.91206	28.35
722	.93918	.93618	.93318	.93015	.92712	.92412	.92100	.91785	.91471	28.43
724	.94188	.93887	.93586	.93284	.92982	.92676	.92371	.92056	.91741	28.50
726	.94459	.94159	.93859	.93556	.93253	.92944	.92635	.92323	.92012	28.58
728	.94735	.94435	.94135	.93830	.93524	.93215	.92906	.92591	.92276	28.66
730	.95006	.94706	.94406	.94103	.93800	.93488	.93176	.92861	.92547	28.74
732	.95282	.94979	.94676	.94373	.94071	.93759	.93447	.93132	.92819	28.82
734	.95553	.95250	.94947	.94644	.94341	.94034	.93718	.93403	.93088	28.90
736	.95824	.95521	.95218	.94915	.94612	.94300	.93988	.93670	.93353	28.98
738	.96100	.95797	.95494	.95188	.94882	.94570	.94259	.93941	.93624	29.06
740	.96371	.96068	.95765	.95459	.95153	.94841	.94529	.94211	.93894	29.13
742	.96647	.96341	.96035	.95730	.95424	.95112	.94800	.94482	.94165	29.21
744	.96918	.96613	.96312	.96003	.95694	.95382	.95071	.94750	.94429	29.29
746	.97188	.96885	.96582	.96273	.95965	.95653	.95341	.95020	.94700	29.37
748	.97465	.97159	.96853	.96544	.96235	.95925	.95606	.95288	.94971	29.45
750	.97735	.97429	.97124	.96815	.96506	.96191	.95876	.95558	.95241	29.53
752	.98006	.97703	.97400	.97088	.96776	.96461	.96147	.95826	.95506	29.61
754	.98282	.97976	.97671	.97359	.97047	.96732	.96418	.96097	.95776	29.69
756	.98553	.98247	.97941	.97629	.97318	.97003	.96688	.96367	.96047	29.76
758	.98829	.98521	.98212	.97900	.97588	.97273	.96959	.96638	.96318	29.84
760	.99100	.98794	.98488	.98176	.97865	.97547	.97229	.96908	.96588	29.92
762	.99371	.99065	.98759	.98443	.98135	.97817	.97500	.97176	.96853	30.00
764	.99647	.99338	.99029	.98717	.98406	.98088	.97771	.97447	.97124	30.08
766	.99918	.99609	.99300	.98988	.98676	.98356	.98035	.97714	.97394	30.16
768	1.00188	.99880	.99571	.99259	.98947	.98629	.98312	.97986	.97660	30.24
770	.00465	1.00156	.99847	.99532	.99218	.98897	.98576	.98252	.97929	30.31
°F.	87.8°	88.7°	89.6°	90.5°	91.4°	92.3°	93.2°	94.1°	95.0°	

## APPENDIX 1. STANDARD SOLUTIONS—TENTATIVE

### SODIUM HYDROXIDE<sup>1</sup>

1

#### APPARATUS

The buret and pipet used should be Bureau of Standards calibrated or should be calibrated by the analyst. Automatic burets should have all exits to the air protected from CO<sub>2</sub> contamination by suitable guard tubes containing soda-lime. All containers should be of alkali-resisting glass.

2

#### REAGENTS

(a) *Carbonate-free H<sub>2</sub>O*.—Prepare by one of the following methods: (1) Boil distilled H<sub>2</sub>O 20 min. and cool with soda-lime protection; (2) bubble air, freed from CO<sub>2</sub> by passing thru a tower of soda-lime, thru distilled H<sub>2</sub>O for 12 hours.

(b) *1+1 alkali*.—To one part of NaOH (reagent quality containing less than 5% Na<sub>2</sub>CO<sub>3</sub>) in a flask add one part of distilled H<sub>2</sub>O and swirl until solution is complete. Close with a rubber stopper. Set aside until Na<sub>2</sub>CO<sub>3</sub> has settled, leaving a perfectly clear liquid (about 10 days).

(c) *Potassium acid phthalate*.—U. S. Bureau of Standards Sample Standard for Acidimetry. Dry for 2 hours at 120°. Cool in a desiccator containing H<sub>2</sub>SO<sub>4</sub>.

(d) *Phenolphthalein indicator*.—1.0 g in 100 cc of 95% alcohol.

3

#### PREPARATION OF STANDARD SOLUTION

The following table gives the approximate amount of 1+1 alkali necessary to make 10 liters of standard soln:

<i>Approx. normality</i>	<i>1+1 alkali to be diluted to 10 liters (cc)</i>
0.01	5.4
0.02	10.8
0.10	54.0
0.50	270.0
1.0	540.0

Add the required amount of 1+1 alkali to 10 liters of CO<sub>2</sub>-free H<sub>2</sub>O. Check the normality, which should be slightly strong, as directed under 4, and adjust to desired strength by the following formula:  $V_1 = V_2 \times N_2 / N_1$ , where  $N_2$  and  $V_2$  represent the normality and volume of stock soln, respectively, and  $V_1$  the volume to which the stock soln should be diluted to obtain the desired normality,  $N_1$ . Determine the exact strength of the final soln as directed under 4.

4

#### STANDARDIZATION<sup>1</sup>

Accurately weigh sufficient dried acid potassium phthalate to titrate approximately 40 cc and transfer to a 300 cc flask that has been swept free from CO<sub>2</sub>. Add 50 cc of cool CO<sub>2</sub>-free H<sub>2</sub>O. Stopper the flask and swirl gently until the sample is dissolved. Add 3 drops of the phenolphthalein indicator and titrate with the soln that is being standardized.

Calculate the normality ( $N$ ) of the standard soln by the following formula:

$$N = \frac{\text{g potassium acid phthalate}}{\text{cc NaOH} \times 204.136/1000}$$

(The normality value is exact only when phenolphthalein is used as an indicator.)

## METHODS OF ANALYSIS

### HYDROCHLORIC ACID

5

#### PREPARATION OF STANDARD SOLUTIONS

The following table gives the approximate amount of HCl (reagent quality, 35-37% HCl) necessary to make 10 liters of standard solns:

<i>Approx. normality</i>	<i>HCl to be diluted to 10 liters (cc)</i>
0.01	8.9
0.02	17.8
0.10	89.0
0.50	445.0
1.0	890.0

6

#### STANDARDIZATION

Titrate 40 cc against a standard alkali soln of approximately the same strength as the acid being standardized as directed under 4, using phenolphthalein as an indicator, 2(d). Determine the normality by the following formula:

$$N = \frac{\text{cc standard alkali} \times \text{normality of alkali}}{\text{cc HCl}}$$

If stronger than desired, dilute the soln to a definite normality value by the following formula:

$$V_1 = \frac{V_2 \times N_2}{N_1}, \text{ where}$$

$N_2$  and  $V_2$  represent the normality and volume of stock soln, respectively, and  $V_1$  represents the volume to which the stock soln should be diluted to obtain the desired normality,  $N_1$ .

Check the exact strength of the final soln by titration as directed above. The normality will be exact only if the same indicator is used in a determination and in the standardization.

If the standard acid soln is to be used with methyl orange as an indicator, determine a correction for the volume of acid required to pass from the end point of phenolphthalein to that of methyl orange. Add<sup>2</sup> 3 drops of a 1% soln of phenolphthalein to 100 cc of  $\text{CO}_2$ -free  $\text{H}_2\text{O}$ , and then add sufficient alkali soln to give an end point with phenolphthalein. Disregard the quantity of alkali soln added and take the buret readings from this point. Add 3 drops of a 0.02% soln of methyl orange and sufficient 0.1  $N$  acid to produce the pink color of methyl orange. Titrate back with 0.1  $N$  alkali soln to the same end point taken in the usual titration (preferably pH = 4.2). Buffered solns of 3.8, 4.0, and 4.2 pH are useful in accurately determining the methyl orange end point. They may be prepared as follows:<sup>2</sup>

pH = 3.8, 2.041 g KH phthalate + 5.26 cc 0.1  $N$  HCl. Dilute to 200 cc.

pH = 4.0, 2.041 g KH phthalate + 0.80 cc 0.1  $N$  NaOH. Dilute to 200 cc.

pH = 4.2, 2.041 g KH phthalate + 7.40 cc 0.1  $N$  NaOH. Dilute to 200 cc.

If the acid and alkali solns are equivalent, the quantity of acid—the quantity of alkali soln = the quantity of acid required to pass from the phenolphthalein end point to that of methyl orange.

#### SELECTED REFERENCES

<sup>1</sup> J. Assoc. Official Agr. Chem., 19, 107 (1935).

<sup>2</sup> Clark, Determination of Hydrogen Ions, 2nd ed., p. 106 (1922).

## APPENDIX 2. DEFINITIONS OF TERMS AND INTERPRETATIONS OF RESULTS ON FERTILIZERS AND LIMING MATERIALS

### DEFINITIONS

#### ACIDULATED FISH TANKAGE, ACIDULATED FISH SCRAP

*Acidulated fish tankage, acidulated fish scrap*, is the rendered product derived from fish and treated with sulfuric acid (adopted 1930).

#### ACTIVATED SEWAGE PRODUCTS

*Activated sewage products* are those made from sewage freed from grit and coarse solids and aerated after being inoculated with microorganisms. The resulting flocculated organic matter is withdrawn from the tanks, filtered with or without the aid of coagulants, dried, ground, and screened (adopted 1930).

#### AGRICULTURAL LIMING MATERIAL

*Agricultural liming material* is material whose calcium and magnesium content is capable of neutralizing soil acidity (adopted 1935).

#### AIR-SLAKED LIME

*Air-slaked lime* is the product obtained by exposing quicklime or hydrated lime to the atmosphere until partly carbonated (adopted 1931, withdrawn for amendment, 1935).

#### AMMONIATED SUPERPHOSPHATE

*Ammoniated superphosphate* is a product containing superphosphate and/or dissolved bone and nitrogenous compounds, but without the addition of potash (adopted 1930).

#### ANALYSIS

The word *analysis*, as applied to fertilizers, shall designate the percentage composition of the product expressed in terms of nitrogen, phosphoric acid, and potash in their various forms (adopted 1926).

#### ASHES FROM LEACHED WOOD

*Ashes from leached wood* are unleached ashes resulting from burning wood that has been exposed to or digested in water or other liquid solvent, as in the extraction of dyes, so that a part of the plant food has been dissolved and removed (adopted 1926).

#### AVAILABLE PHOSPHORIC ACID

*Available phosphoric acid* is the sum of the water-soluble and the citrate-soluble phosphoric acid (adopted 1931).

#### "BASIC" LIME PHOSPHATE

*"Basic" lime phosphate (lime-based superphosphate)* is a superphosphate to which liming materials have been added in a quantity at least six per cent (6%) calcium carbonate equivalents in excess of the quantity required to convert all water-soluble phosphate to the citrate-soluble form (adopted 1934).

## METHODS OF ANALYSIS

### BASIC PHOSPHATE SLAG

*Basic phosphate slag* is a by-product in the manufacture of steel from phosphatic iron ores. The product shall be finely ground and shall contain no admixture of materials other than what results in the original process of manufacture. It shall contain not less than twelve per cent (12%) of total phosphoric acid ( $P_2O_5$ ), not less than eighty per cent (80%) of which shall be soluble in two per cent (2%) citric acid solution according to the Wagner method of analysis. Any phosphate slag not conforming to this definition shall be designated *low grade* (adopted 1925).

### BRAND AND BRAND NAME

A *brand* is a term, design, or trademark used in connection with one or several grades of fertilizers (adopted 1926).

A *brand name* is a specific designation applied to an individual fertilizer (adopted 1926).

### CITRATE-SOLUBLE ("REVERTED") PHOSPHORIC ACID

*Citrate-soluble ("reverted") phosphoric acid* is that part of the total phosphoric acid in a fertilizer that is insoluble in water but soluble in a solution of citrate of ammonia according to the method adopted by the A.O.A.C. (adopted 1932).

### CRUDE, INERT OR SLOW-ACTING NITROGENOUS MATERIALS

*Crude, inert, or slow-acting nitrogenous materials* are unprocessed organic substances relatively high in nitrogen but having a very low value as plant food and showing a low activity by both the alkaline and neutral permanganate methods below 50% and 80%, respectively (adopted 1929).

### CYANAMID

*Cyanamid* is a commercial product composed chiefly of calcium cyanamid ( $CaCN_2$ ), and it shall contain not less than twenty-one per cent (21%) of nitrogen (adopted 1935).

### DICALCIUM PHOSPHATE

*Dicalcium phosphate* is a manufactured product consisting chiefly of a dicalcic salt of phosphoric acid (adopted 1931).

### DISSOLVED BONE

*Dissolved bone* is ground bone or bone meal that has been treated with sulfuric acid (adopted 1926).

### DRIED BLOOD

*Dried blood* is the collected blood of slaughtered animals, dried and ground and containing not less than twelve per cent (12%) of nitrogen in organic forms (adopted 1928).

### DRIED, PULVERIZED, OR SHREDDED MANURES

*Dried, pulverized, or shredded manures* are what the name indicates, and not mixtures of manures and other materials (adopted 1925).

### FERTILIZER GRADE

*Fertilizer grade* shall represent the minimum guaranty of its plant food expressed in terms of *nitrogen (not ammonia)*, *available phosphoric acid*, and *water-soluble potash* (adopted 1928).

## APPENDIX

### FISH TANKAGE, FISH SCRAP, DRY GROUND FISH, FISH MEAL FERTILIZER GRADE

*Fish tankage, fish scrap, dry ground fish, fish meal fertilizer grade*, is the dried ground product derived from rendered or unrendered fish (adopted 1929).

### GARBAGE TANKAGE

*Garbage tankage* is the rendered, dried, and ground product derived from waste household food materials (adopted 1929).

### GROUND LIMESTONE

*Ground limestone* is the product obtained by grinding calcareous or dolomitic limestone. Not less than seventy-five per cent (75%) shall pass a 100-mesh sieve. It shall contain calcium and magnesium carbonates equivalent to not less than ninety per cent (90%) of calcium carbonate (adopted 1931, withdrawn for amendment, 1935).

### GROUND RAW BONE

*Ground raw bone* is dried ground animal bones that have not been previously steamed under pressure (adopted 1929).

### GROUND SHELL LIME

*Ground shell lime* is the product obtained by grinding the shells of mollusks. Not less than seventy-five per cent (75%) shall pass a 100-mesh sieve. It shall contain calcium and magnesium carbonates equivalent to not less than eighty per cent (80%) of calcium carbonate (adopted 1931, withdrawn for amendment, 1935).

### GROUND SHELL MARL

*Ground shell marl* is the product obtained by grinding natural deposits of shell marl. Not less than seventy-five per cent (75%) shall pass a 100-mesh sieve. It shall contain calcium and magnesium carbonates equivalent to not less than eighty per cent of calcium carbonate (adopted 1931, withdrawn for amendment, 1935).

### GROUND STEAMED BONE

*Ground steamed bone* is ground animal bones that have been previously steamed under pressure (adopted 1929).

### GYPNUM, LAND PLASTER, OR CRUDE CALCIUM SULFATE

*Gypsum, land plaster, or crude calcium sulfate* are products consisting chiefly of calcium sulfate. They may contain twenty per cent (20%) of combined water. (They do not neutralize acid soils) (adopted 1931).

### HIGH CALCIC PRODUCTS

*High calcic products* are materials of which 90% or more of the total calcium and magnesium content consists of calcium oxide (adopted 1935).

### HIGH MAGNESIC PRODUCTS

*High magnesian products* are materials in which more than 10 per cent of the total calcium and magnesium oxide consists of magnesium oxide (adopted 1935).

### HOOF AND HORN MEAL

*Hoof and horn meal* is processed dried, ground hoofs and horns (adopted 1929).



## METHODS OF ANALYSIS

### HYDRATED OR SLAKED LIME

*Hydrated or slaked lime* is a dry product consisting chiefly of the hydroxide of calcium and oxide-hydroxide of magnesium (adopted 1935).

### KAINIT

*Kainit* is a potash salt containing potassium and sodium chlorides and sometimes sulfate of magnesia with not less than twelve per cent (12%) of potash ( $K_2O$ ) (adopted 1928).

### LEACHED WOOD ASHES

*Leached wood ashes* are ashes from burned unleached wood with part of their plant food removed by artificial means or by exposure to rains, snows, or other solvent (adopted 1928).

### LIME

The word *lime* when applied to liming materials means either calcium oxide or calcium and magnesium oxides (adopted 1934).

### MANGANESE

*Manganese*. The Committee recommends that either the water-soluble or available manganese in fertilizers be expressed as manganese (Mn) (adopted 1935).

### MANGANESE SULFATE

*Manganese sulfate*. The term manganese sulfate, when applied to an ingredient of a mixed fertilizer, shall designate anhydrous manganous sulfate ( $MnSO_4$ ) (adopted 1935).

### MANURE SALTS

*Manure salts* are potash salts containing high percentages of chloride and from twenty per cent (20%) to thirty per cent (30%) of potash ( $K_2O$ ). The term *double manure salts* should be discontinued (adopted 1925).

### MONO-AMMONIUM PHOSPHATE (FERTILIZER GRADE)

*Monoammonium phosphate (fertilizer grade)* is a commercial salt made by combining phosphoric acid with ammonia. It shall contain not less than ten per cent (10%) of nitrogen and not less than forty-six per cent (46%) of available phosphoric acid (adopted 1934).

### MURIATE OF POTASH (COMMERCIAL POTASSIUM CHLORIDE)

*Muriate of potash* is a potash salt containing not less than forty-eight per cent (48%) of potash ( $K_2O$ ), chiefly as chlorides (adopted 1929).

### NITRATE OF POTASH (COMMERCIAL POTASSIUM NITRATE)

*Nitrate of potash* is a salt containing not less than twelve per cent (12%) of nitrogen and forty-four per cent (44%) of potash ( $K_2O$ ) (adopted 1927).

### NITRATE OF SODA (COMMERCIAL SODIUM NITRATE)

*Nitrate of soda* is commercial sodium nitrate containing not less than fifteen per cent (15%) of nitrogen, chiefly as sodium nitrate (adopted 1928).

## APPENDIX

### PEAT

*Peat* is partly decayed vegetable matter of natural occurrence. It is composed chiefly of organic matter which contains some nitrogen of low activity (adopted 1931).

### CHARRED PEAT

*Charred peat* is peat artificially dried at a temperature that causes partial decomposition (adopted 1931).

### PHOSPHATE ROCK

*Phosphate rock* is a natural rock containing one or more calcium phosphate minerals of sufficient purity and quantity to permit its use, either directly or after concentration, in the manufacture of commercial products (adopted 1933).

### PHOSPHORIC ACID

The term *phosphoric acid* designates phosphorus pentoxide ( $P_2O_5$ ) (adopted 1934).

### POTASH

The term *potash* designates potassium oxide ( $K_2O$ ) (adopted 1934).

### PRECIPITATED BONE PHOSPHATE

*Precipitated bone phosphate* is a by-product from the manufacture of glue from bones and is obtained by neutralizing the hydrochloric acid solution of processed bone with calcium hydroxide. The phosphoric acid is chiefly present as dicalcium phosphate (adopted 1933).

### PRECIPITATED PHOSPHATE

*Precipitated phosphate* is a product consisting mainly of dicalcium phosphate obtained by neutralizing with calcium hydroxide the acid solution of either phosphate rock or processed bone (adopted 1933).

### PROCESS TANKAGES

*Process tankages* are products made under steam pressure from crude inert nitrogenous materials, with or without the use of acids, for the purpose of increasing the activity of the nitrogen. These products shall be called "Process Tankages" with or without further qualification. The water-insoluble nitrogen in these products shall test at least fifty per cent (50%) active by the alkaline, or eighty per cent (80%) by the neutral permanganate method (adopted 1931).

### PRODUCTS SECURED BY HEATING CALCIUM PHOSPHATE WITH ALKALI SALTS CONTAINING POTASH

*Products secured by heating calcium phosphate with alkali salts containing potash* are non-acid phosphates with potash. They are *not* potassium phosphate (adopted 1928).

### QUICK LIME, BURNED LIME, CAUSTIC LIME, LUMP LIME, UNSLAKED LIME

*Quick lime, burned lime, caustic lime, lump lime, unslaked lime.* These designations shall apply to calcined materials, the major part of which is calcium oxide, in natural association with a lesser amount of magnesium oxide, and which is capable of slaking with water (adopted 1935).

## METHODS OF ANALYSIS

### SHEEP MANURE—WOOL WASTE

*Sheep manure—wool waste* is the by-product from wool-carding establishments consisting chiefly of sheep manure, seeds, and wool fiber (adopted 1931).

### SOFT PHOSPHATE WITH COLLOIDAL CLAY

*Soft phosphate with colloidal clay* is a very finely divided low-analysis by-product from mining Florida rock phosphate by a hydraulic process in which the colloidal materials settle at points in artificial ponds and basins farthest from the washer, and are later removed after the natural evaporation of the water (adopted 1933).

### SULFATE OF AMMONIA (COMMERCIAL AMMONIUM SULFATE)

*Sulfate of ammonia (commercial ammonium sulfate)* is a commercial product composed chiefly of ammonium sulfate. It shall contain not less than twenty and five-tenths per cent (20.5%) of nitrogen (adopted 1931).

### SULFATE OF POTASH-MAGNESIA

*Sulfate of potash-magnesia* is a potash salt containing not less than twenty-five per cent (25%) of potash ( $K_2O$ ), nor less than twenty-five per cent (25%) of sulfate of magnesia, and not more than two and one-half per cent (2.5%) of chlorine (adopted 1925).

### SULFATE OF POTASH (COMMERCIAL POTASSIUM SULFATE)

*Sulfate of potash (commercial potassium sulfate)* is a potash salt containing not less than forty-eight per cent (48%) of potash ( $K_2O$ ) chiefly as sulfate, and not more than two and one-half per cent (2.5%) of chlorine (adopted 1929).

### SUPERPHOSPHATE

*Superphosphate* is the cured product obtained by mixing rock phosphate with sulfuric or phosphoric acid or with both (adopted 1933, withdrawn for amendment, 1935).

### TANKAGE

*Tankage* (without qualification) is the rendered, dried, and ground by-product, largely meat and bone from animals (slaughtered or that have died otherwise) (adopted 1929).

### UNIT OF PLANT FOOD

A *unit of plant food* is twenty (20) pounds, or one per cent (1%) of a ton (adopted 1926).

### UNLEACHED WOOD ASHES

*Unleached wood ashes* are ashes from burned unleached wood that have had no part of their plant food removed and that contain four per cent (4%) or more of water-soluble potash ( $K_2O$ ), (adopted 1928).

### WASTE LIME, BY-PRODUCT LIME

*Waste lime, by-product lime*, is any industrial waste or by-product containing calcium or calcium and magnesium in forms that will neutralize acids. It may be designated by the prefixation of the name of the industry or process by which it is produced, i.e., gas-house lime, tanners' lime, acetylene lime-waste, lime-kiln ashes, calcium silicate, etc. (adopted 1931).

## APPENDIX

### INTERPRETATIONS

#### ACTIVITY OF WATER-INSOLUBLE NITROGEN IN MIXED FERTILIZERS

*Activity of water-insoluble nitrogen in mixed fertilizers.* The alkaline and neutral permanganate methods distinguish between the better and the poorer sources of water-insoluble nitrogen, and do not show the percentage availability of the materials. The available nitrogen of any product can be measured only after carefully conducted vegetation experiments.

(a) The methods shall be used on mixed fertilizers containing water-insoluble nitrogen amounting to three-tenths of one per cent (0.3%) or more of the weight of the material. If a total nitrogen exceeds the minimum guaranty and is accompanied by a low activity of the insoluble nitrogen, the over-run shall be taken into consideration in determining the classification of the water-insoluble nitrogen.

(b) The water-insoluble nitrogen in mixed fertilizers showing an activity below fifty per cent (50%) by the alkaline method and also below eighty per cent (80%) by the neutral method shall be classed as inferior. This necessitates the use of both methods, also the provision as to over-run in (a), before classifying as inferior (adopted 1927).

#### AMOUNT OF CHLORINE PERMISSIBLE IN FERTILIZERS IN WHICH THE POTASH IS CLAIMED AS SULFATE

*Amount of chlorine permissible in fertilizers in which the potash is claimed as sulfate.* The chlorine in mixed fertilizers in which the potash is claimed as sulfate shall not exceed one-half of one per cent (0.5%) more than what is called for in the minimum potash content based on the definition of sulfate of potash as formulated by the Committee. Calculate as follows: 0.05 times the percentage of potash found plus 0.5 (adopted 1928).

#### BRAND NAME TO INCLUDE ANALYSIS OR GRADE OF FERTILIZER

*The analysis or grade of a fertilizer* should be included with its brand name, and so used by the manufacturer on sacks and in printed literature and by the control official in his reports and publications (adopted 1927).

#### CYANAMIDE AND UREA NITROGEN

*Cyanamide and urea nitrogen* is synthetic non-protein organic nitrogen (adopted 1931).

#### FERTILIZER FORMULA

*A fertilizer formula* shall express the quantity and grade of the crude stock materials used in making a fertilizer mixture. For example: 800 pounds of 16% superphosphate, 800 pounds of tankage (7.40 nitrogen and 9.15 total phosphoric acid), and 400 pounds of sulfate of potash-magnesia (twenty-six per cent (26%) potash) (adopted 1926).

#### FINELY GROUND AS APPLIED TO BASIC PHOSPHATE SLAG

*Finely ground* in the definition of basic phosphate slag shall refer to actual size of particles as determined by the use of standard sieves, as follows: seventy per cent (70%) or more shall pass a 100-, and ninety per cent (90%) or more shall pass a 50-mesh sieve (adopted 1927).

## METHODS OF ANALYSIS

### THE WORD "LIME" AS APPLIED TO FERTILIZERS

The term "*lime*" shall not be used in the registration, labelling, or guaranteeing of fertilizers or fertilizing materials unless the lime is in a form or forms to neutralize soil acidity (adopted 1935).

### NET WEIGHTS

The weights appearing on packages of fertilizer, agricultural lime, and liming materials shall always mean *net weights* (adopted 1932).

### ORDER OF TERMS

The *order of terms* in mixed fertilizers shall be nitrogen first, phosphoric acid second, and potash third (adopted 1930).

### NAME OF A FERTILIZER MATERIAL USED AS THE BRAND NAME OR PART OF THE BRAND NAME OF A MIXED FERTILIZER

When the name of a fertilizer material is used as a part of the brand name of a mixed fertilizer, as for example, blood, bone, or fish, the nitrogen or phosphoric acid shall be derived from or supplied entirely by the material named. When the name of a fertilizer material is used as a brand or as part of a brand and the nitrogen or phosphoric acid is not supplied by the material named, the word "brand" shall follow the name of the materials. Example: "Fish Brand Fertilizer" (adopted 1930).

### STATEMENT OF GUARANTIES

The *statement of guaranties* of mixed fertilizers shall be given in whole numbers (adopted 1930).

### UNIFORMITY IN USE OF TERMS "PHOSPHORIC ACID" AND "POTASH"

As the terms *phosphoric acid* and *potash* are used universally in guaranteeing and in reporting the analyses of fertilizers, it is recommended that the same terms also be used in reporting and discussing the results of analyses of related materials (adopted 1934).

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